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Journal of DENTAL RESEARCH

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HAMILTON B. G. ROBINSON

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Objects (Const., Art. II): . . ."(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

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EDITOR'S VIEWPOINT

Crowell (p. 592) examined the metallography of dental amalgam alloys by the etching technic. This study is presented as a series of photomicrographs showing the results.

Dewar and Parfitt (p. 596) measured certain physical properties and the mucin content of 700 saliva samples. They point out the need for precaution in collecting and handling the samples. Activated saliva showed lower mucin content than resting saliva, suggesting that the mucin may not be a product of salivary glands or that the method of measuring tyrosine may not be applicable when bacteria and other cells are introduced from the mouth. Some relationship between viscosity, ropiness, and content of polymerized mucin was observed.

Muhler, Radike, Nebergall, and Day (p. 606) made a clinical test of a dentifrice containing stannous fluoride. Laboratory studies with tin-fluoride combinations have suggested that this compound may be superior to sodium fluoride in reducing dental caries activity. Results at the end of six months are encouraging but not conclusive. It is interesting that the 52 per cent reduction (by DMF) or the 72 per cent reduction (by DMF surfaces) is accounted for chiefly through reduction in interproximal caries. Additional results from this study should be interesting and those, to date, should encourage further study. Will there be a "therapeutic dentifrice"?

Grainger and Reid (p. 613) examined, statistically, the data on frequency distribution of caries in 1,200 school children. While negative binominal distribution described the smooth surface lesions, no simple probability distribution fit the "pitted" surface. They propose that differing individual susceptibilities are important in determining this chance process of dental caries distribution.

It has been rather generally accepted that bacteria are highly important in producing gingivitis, but the absence of bacteria within some inflamed gingival tissues has puzzled a few workers. Schultz-Haudt, Bibby, and Bruce (p. 624) used bacteria-free extracts from gingival deposits of patients with and without gingivitis. In the extracts from gingivitis, enzymes capable of attacking hyaluronic acid, chondroitin sulfate, and collagen were demonstrated, although they appeared absent or inactive in gingivitis-free cases. They also found microorganisms from gingival pockets to be capable of producing hyaluronidase and collagenase. These demonstrations serve to emphasize the role of bacteria as chemical factories producing destructive enzymes.

Riedesel and Fisher (p. 632), from analysis of the nitrogen content of bovine pulps, human infant, and adult pulps, found species differences but no age differences. Their values are higher than previously reported. Preservation in 10 per cent formalin did not appear to affect the analysis.

The Proceedings of the 1954 meeting of the International Association for Dental Research are included in this issue (p. 637). The meeting is held in March and delays in receiving revised abstracts, the time required to process the manuscripts, and for the printer to set up type, proof to be read, and corrections made, occupied too many weeks to permit publication in the June or August issue. The Association is making an effort to require submission of final abstracts by December 1, and is considering prepublication of abstracts. The 124 items presented at the General Sessions, the thirty-one read at the Material Group Sessions, and the forty-six presented "by title" afford a review of dental research activities for 1953-1954.

The British Section of the International Association for Dental Research appears to be holding important annual sessions which are growing in scope and depth (p. 729). This increasing activity is a most welcome sign and we hope that others of our Sections abroad will present comparable programs.

H. B. G. R.

THE METALLOGRAPHY OF DENTAL AMALGAM ALLOYS

WALTER S. CROWELL

Philadelphia, Pa.

AS A step in correlating the chemical composition of dental amalgam alloys with the physical properties of the amalgams made from them, a study was made of the metallography of amalgam alloys.

Generally speaking, dental amalgam alloys are alloys of silver, copper, tin, and zinc. Silver and tin are the major constituents. All of the binary systems and the ternary system copper-tin-zinc have been investigated, and their constitution is well understood.[1] As far as we know, there is no published work on the ternary system silver-copper-tin, nor on these alloys containing small quantities of zinc.

When the formulas of amalgam alloys, which have useful properties, were calculated to molecular percentage of silver, copper, and tin, it was found that they run from 22 to 25 atom per cent tin and from 2 to 8 atom per cent copper, the remainder being silver. The metallography of this area of composition was studied by preparing a series of samples in which the tin was varied at a constant copper level of 4 atom per cent and another series in which the copper was varied at a constant tin level of 24 per cent. In both series 1 per cent by weight of zinc was added "outside the formula" to ensure sound ingots.

The specimens used were sections of ingots weighing about ten ounces. They were annealed twenty-four hours at 400° C. before they were prepared for metallographic examination.

While the work was in process, a sample containing no copper was added to the 24 per cent tin series. A sample was available which was added to the 4 atom per cent copper series. It contained 26 atom per cent tin and 1.8 weight per cent zinc. Alloy No. A-3 was made for another purpose. It contains 23.8 atom per cent tin and 4.2 atom per cent copper. The atomic and weight percentage compositions are given in Table I.

TABLE I

NO.	ATOM PER CENT			WEIGHT PER CENT			
	Ag	Cu	Sn	Ag	Cu	Sn	Zn
A-1	76	0	24	73.4	0	25.6	1
A-2	74	2	24	72.1	1.2	25.7	1
A-3	72	4.2	23.8	70.9	2.4	25.7	1
A-4	70	6	24	69.3	3.5	26.2	1
A-5	68	8	24	67.9	4.7	26.4	1
A-6	74	4	22	72.8	2.3	23.9	1
A-7	73	4	23	71.8	2.3	24.9	1
A-8	71	4	25	69.7	2.3	27.0	1
A-9	70	4	26	68.0	2.3	27.8	1.8

The etching of the specimens prepared for metallographic examination presented some difficulties. In his investigation of the alloys of silver and tin,

Presented at the meeting of the Materials Group, International Association for Dental Research, Philadelphia, Pennsylvania, March 20-22, 1953 (J. D. Res. 32: 713, 1953).

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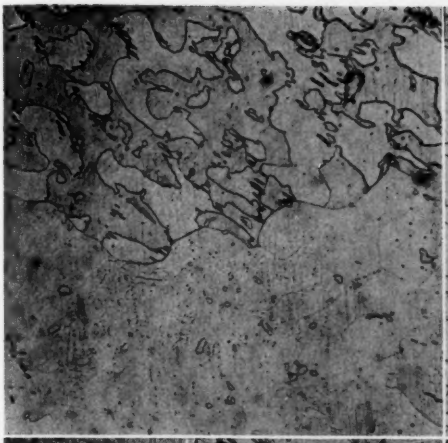
Fig. 1.



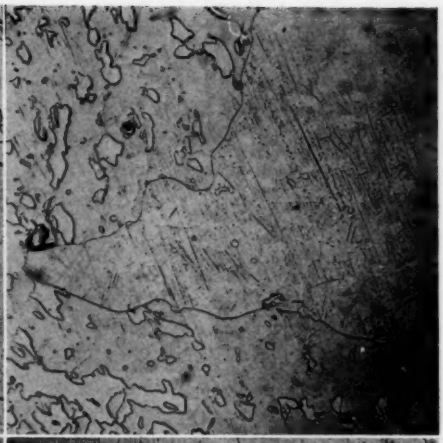


Fig. 2.

Fig. 3.

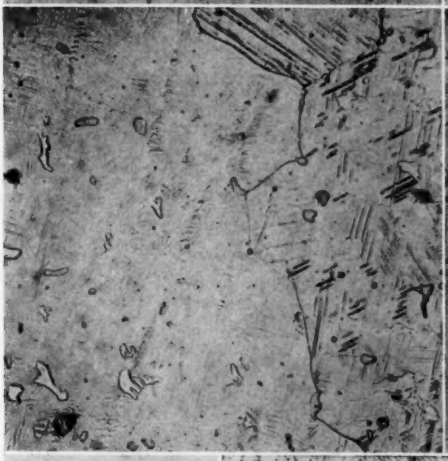


Fig. 4.

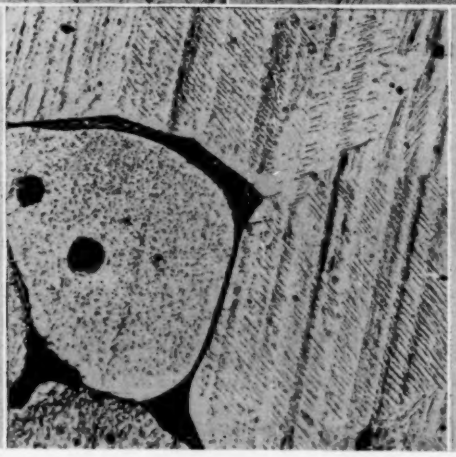


Fig. 5.

Fig. 1.—Sn 22, Cu 4.
Fig. 2.—Sn 23, Cu 4.
Fig. 3.—Sn 23.8, Cu 4.2.
Fig. 4.—Sn 25, Cu 4.
Fig. 5.—Sn 26, Cu 4.

Murphy[2] found that electrolytic etching in 1 per cent hydrofluoric acid gave good results. When etched by this method, it was very difficult to obtain evenly etched, unpitted, unstained sections when the alloy contained 1 atom per cent or more of copper. A modification of the well-known chromic acid etching

Fig. 6. Fig. 7.

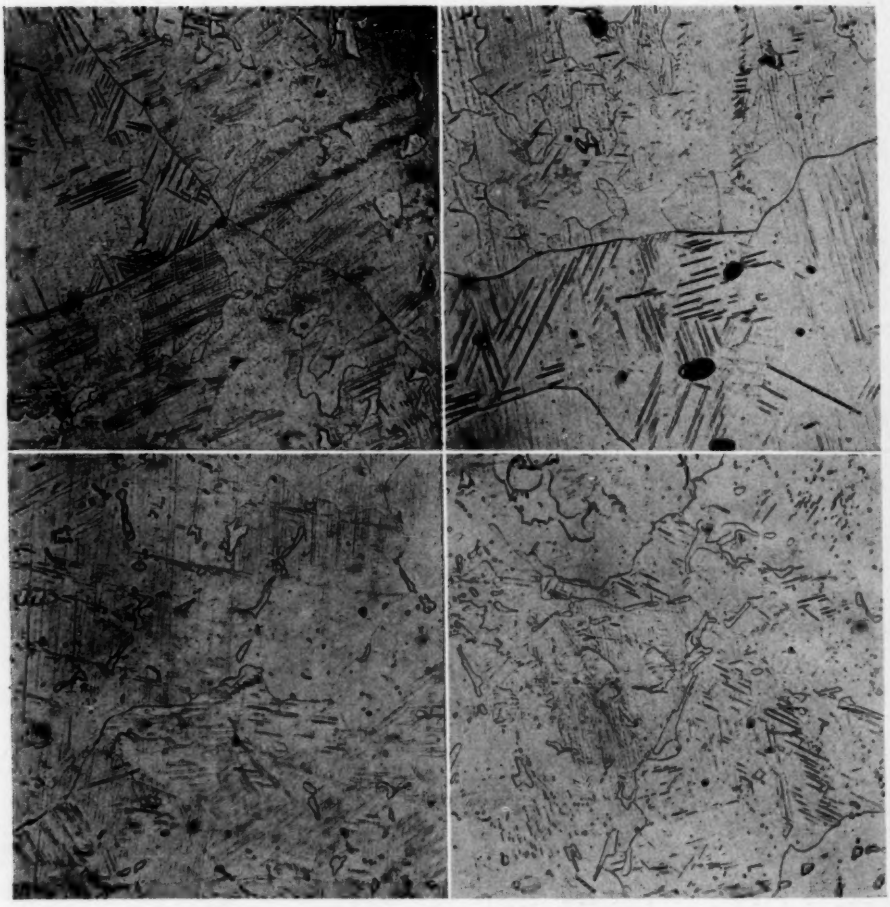


Fig. 8. Fig. 9.

Fig. 6.—Sn 24, Cu O.
Fig. 7.—Sn 24, Cu 2.
Fig. 8.—Sn 24, Cu 6.
Fig. 9.—Sn 24, Cu 8.

solution used for copper-tin alloys gave excellent results. The formula is as follows:

$K_2Cr_2O_7$	2 Gm.
NaF	1 Gm.
H_2SO_4 concentrated specific gravity 1.84	3 ml.
H_2O	100 ml.

The etching solution for bronze is activated by adding a small quantity of sodium chloride. It will etch the silver-copper-tin-zinc alloys also, but the surface of the specimen is covered with a film of silver chloride which must be removed by sodium thiosulfate or ammonia before the structure can be studied. When sodium fluoride is substituted for sodium chloride, no deposit is formed, and the specimen is ready for examination after washing with water and drying with alcohol in the usual way.

Etching proceeds at a moderate speed. With the relatively coarse-grained alloys we were studying, it could be followed visually. Usually thirty seconds at room temperature produced a very satisfactory etch, which showed grain boundaries and the internal structure of individual grains clearly. The concentration of sulfuric acid is not critical. The quantity given is close to the minimum. Doubling it increases the rate of attack somewhat, but otherwise does not affect the results.

Photomicrographs of the alloys studied are shown in Figs. 1 to 9. All of them are at 500 diameters.

Effect of Increasing Tin at 4 Atom Per Cent Copper (Figs. 1 to 5).— At 22 atom per cent tin, the alloy is duplex, as might be expected from Murphy's[2] work. He found that alloys in this composition range contain the β solid solution and the γ constituent, Ag_3Sn. As tin is increased, the area of the β constituent decreases. At 23.8 atom per cent, it is present in small quantities. At the 4 atom per cent copper level, a third constituent appears as dark etching, rounded grains. At 25 atom per cent tin, the β phase has disappeared but, again at 4 atom per cent copper, the new phase is still present. At 26 atom per cent tin, the eutectic of Ag_3Sn and tin appears between the grains. When annealed at 400° C., this specimen partially melted. Small drops of eutectic appeared on its surface. With the exception of the appearance of the dark etching constituent at 24 and 25 atom per cent tin, these structures coincide with those described by Murphy.

Effect of Increasing Copper at 24 Atom Per Cent Tin (Figs. 3 and 6 to 9).— At 0 and 2 atom per cent copper, these structures are essentially duplex, as described for the silver-tin alloys by Murphy. At 4.2 atom per cent copper, the dark etching phase appears. More of it is present at 6 atom per cent, and still more at 8 atom per cent. The obvious conclusion is that it is copper rich, and probably is the compound Cu_3Sn, which is present in the copper-tin system.

The silver copper system has been thoroughly studied and it is known that the solid solubility of copper in silver is small, amounting to about 2 atom per cent at 400° C. It is reasonable to suppose that Cu_3Sn is equally insoluble in Ag_3Sn. Of course, further evidence will have to be obtained before the composition of the dark etching phase can be considered established.

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1. Metals Handbook, Cleveland, 1948, American Society for Metals.
2. Murphy, A. J.: The Constitution of the Alloys of Silver and Tin, *J. Inst. Metals* **35**: 107, 1926.

AN INVESTIGATION OF THE PHYSICAL PROPERTIES OF SALIVA AND THEIR RELATIONSHIP TO THE MUCIN CONTENT

MARGARET R. DEWAR* AND G. J. PARFITT

Institute of Dental Surgery, University of London, and Eastman Dental Dispensary, Rochester, N. Y.

A NUMBER of studies have been carried out on salivary properties in relation to dental disease, but much of their value is lost as neither the complex nature of saliva in respect to its basic properties nor the actual nature of the physical properties themselves has been fully realized, and in many of them, the methods of collection and treatment of test samples are not given.

Saliva is not a true or Newtonian fluid but possesses properties exhibited by colloidal suspensions and pseudoplastics. True solutions possess only one property of flow, viscosity. The presence of colloidal suspension, however, profoundly alters the properties of the material in which it is suspended and may give rise to properties of flow such as stickiness, adhesiveness, and elasticity. In saliva there are many molecules of various size, some in solution and some in suspension. The interaction of their internal forces results in varied behavior when the saliva is placed under pressure. The actual value of each property varies with the conditions under which the fluid exists and, therefore, when studying the physical properties of nontrue fluids, such as saliva, it is particularly important to know the conditions under which the physical properties are measured.

In spite of the fact that the physical properties of saliva are probably dependent upon the mucin and protein content, it does not seem that a correlated study of the physical properties and the mucin content has been carried out nor has a method for measuring the mucin content of the saliva been established. This paper reports parallel investigations which have been carried out to determine the most practical methods of measuring salivary mucin and the relationship between the mucin content and some measurable physical properties of saliva.

METHODS OF MEASURING SOME OF THE PHYSICAL PROPERTIES OF SALIVA

To measure the properties of saliva, it was necessary to have apparatus capable of being used in a small volume of fluid (1 to 2 ml.), easily cleaned, and quick to use. Various standard types of equipment were tested, but it was found necessary in several instances to devise apparatus for use with saliva.

This paper is based on a portion of a thesis submitted to the University of London, England, in support of the senior author's candidature for the degree of Doctor of Philosophy.

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Viscosity.—Nontrue fluids, such as saliva, display "anomalous viscosity," which means that the rate of flow increases as the pressure rises up to a certain point and, thereafter, the flow decreases. Therefore, when measuring viscosity of saliva, it is necessary to know the pressure at which the measurement of rate of flow is made.

Viscosity at various pressures was measured by means of steel balls traveling down a saliva-filled tube placed at definite angles. A glass tube of 2 mm. bore and stainless steel balls of $\frac{1}{32}$ inch and $\frac{1}{16}$ inch diameter were used. The center five-sevenths of the tube was marked off by two lines, and the time taken for the ball to pass between these two lines was measured. Readings were taken with each sized ball with the tube placed at angles of fifteen degrees, thirty degrees, and forty-five degrees, the viscosity thus being measured under six different pressures designated P_1 to P_6 in increasing magnitude. The apparatus was standardized with glycerol and sucrose solutions. Because changes occur in saliva on standing, only freshly collected saliva is suitable with this technic.

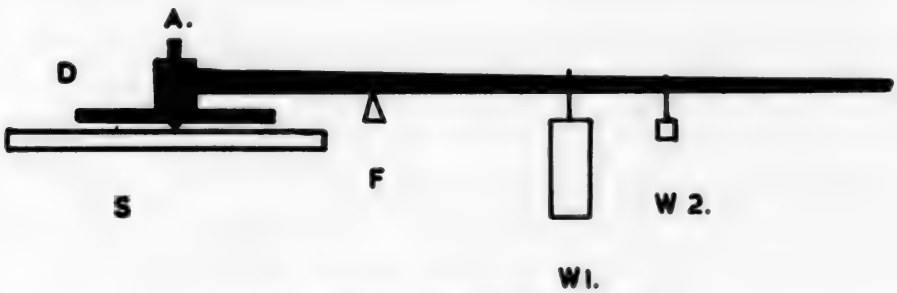

Fig. 1.—Machine used for measuring tack.

Flow elasticity is a property sometimes possessed by fluids in which large molecules are dispersed. When such a fluid is made to flow through a narrow tube under a constant head of pressure, the molecules tend to align themselves and confer, on the fluid medium, properties of flow which are more dependent on the nature of the dispersed molecules than on the medium. When flow is halted, a marked recoil along the tube can be observed.

Flow elasticity was measured by the menstruscope devised by Clift and Scott Blair[1] for use in veterinary work on substances more viscous than average specimens of saliva. Readings could be obtained only on the more highly viscous samples, the ordinary range being quite outside the sensitivity of the instrument.

Tack or tackiness is a measure of adhesiveness or stickiness. It is a surface property which causes two surfaces connected by a layer of fluid to resist separation. Tackiness is usually gauged manually, but a tackmeter for accurate measurement of the tack of printing ink was devised by Green.[2] A simpler machine was devised involving the same principles (Fig. 1). A disc

of 1 sq. cm. (*D*) was made to rest in the fluid to be measured. It was kept in contact with the fluid for ten seconds by a simple weight placed above *A*. When the measurement was to be made, this weight was lifted, and the forces applied at *W 1* (1 Gm.) and *W 2* (0.1 Gm.) were adjusted so that the combined force of *W 1* and *W 2* just exceeded the adhesive force and the disc separated from the saliva. An arbitrary measure of the adhesive force was thus obtained which could be used for comparative purposes. The technic and machine were standardized using egg white and a glycerol-albumin mixture. It was found that the tack of saliva was increased by work hardening, and so the same sample could not be used for repeated readings. Particular care had to be taken to exclude bubbles, as they caused inaccurate readings.

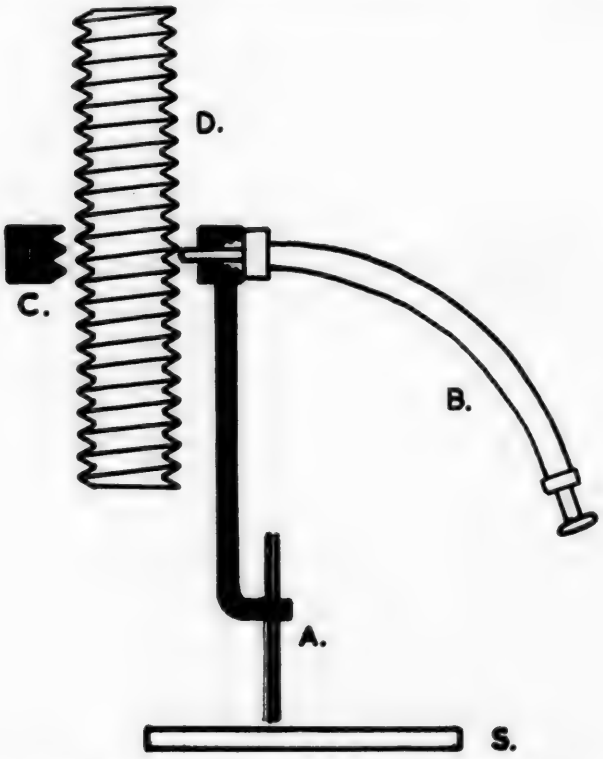

Fig. 2.—Machine used for measuring spinnbarkeit.

Spinnbarkeit.—Some fluids in which large molecules are dispersed can be drawn out into long elastic threads. This property, closely related to "ropiness," is termed "spinnbarkeit." It is thought to be related to flow elasticity and viscosity, but the exact relation has not been determined.

Erbring[3] devised a machine to measure *spinnbarkeit*. This machine used a constant speed motor, a clutch, and a pulley. A simpler apparatus was devised (Fig. 2) involving the same principle as Erbring's machine in that the

fluid to be measured was drawn up at a constant speed until the thread of fluid broke when the clutch (*B*) was immediately released. The distance traveled gave a measure of *spinnbarkeit*. In this machine the vertical pull was exerted by a nut (*C*) traveling up a threaded rod (*D*). Vibration was overcome by special suspension. Constant grip was obtained on the fluid to be measured by using a capillary tube as "applicator" (*A*). As the property of *spinnbarkeit* is also affected by work hardening, the sample of known volume was therefore stirred for a period of ten seconds. The capillary applicator filled up to a height of about 2 cm., and this was found suitable to obtain adequate attachment to the sample. A thin-walled capillary tube of 1 to 2 mm. bore, open at both ends, proved most satisfactory. When the capillary tube was emptied and refilled without further stirring of the sample, three consecutive readings could be carried out on the same sample. The apparatus was standardized with egg white.

Surface Tension.—Nontrue fluids as well as true fluids possess surface tension. This was measured by finding the height to which a fluid rises in a capillary tube. The weight of fluid raised is equal to the downward force on the fluid outside the capillary tube. This downward force is actually a downward pull on the surface from the interior of the fluid and is termed "surface tension." The apparatus was standardized using toluene, acetic acid, and chloroform. Care has to be taken when determining the surface tension of salivas by this method because of the presence of varying amounts of suspended material and the heterogeneous nature of the fluid itself.

Specific Gravity.—A quick method of estimating specific gravity on small quantities of fluid, applied by Willsmore[4] to saliva, was used. It consists of placing drops of saliva into chloroform-benzene solutions of known density 1, 1.01, 1.02, 1.03. The drop has equal specific gravity to the fluid in which it neither sinks nor floats. The specific gravity of saliva was found to be constant and unrelated to any other property, all values falling between 1.01 and 1.02. Willsmore[4] also commented on this constancy in specific gravity.

The Mucin Content of Saliva.—Clement[5] has summarized our present knowledge of the composition of salivary mucin by stating that salivary mucin consists of a protein portion and a carbohydrate portion. The protein portion resembles other body proteins in having a representative series of amino acids joined by peptide linkages. The carbohydrate portion is thought to contain two different hexosamine-hexose combinations: (1) consisting probably of d-glucosamine, two acetyl residues, and a polyhydroxy acid (possibly gluconic acid) and (2) consisting of d-glucosamine and di-d-mannose.

Many methods have been used to estimate the mucin present in saliva and in gastric juice. Some investigators have estimated the carbohydrate part of the molecule, others the protein.

After investigating the various methods, we selected Karshan's[6] modification of Greenberg's tyrosine estimation as the most satisfactory for measuring the mucin content of saliva, uncentrifuged saliva being used in all estimations.

The Congo red in acid alcohol (A.C.R.A.) test devised by Burnet[7] was also used to give a measure of the "polymerized mucin" present in the saliva. This method depends on the formation of a mucin clot in alcohol and consists of making serial dilutions of the saliva, adding Congo red, and dropping one drop of each dilution into acid alcohol. At the "end point," the drop forms an easily distinguishable globule. This test is simple and quick and has proved most satisfactory when applied to saliva.

Treatment of Salivary Samples.—Because saliva changes on standing and when stirred, it was necessary to develop a standard method of treating the sample after collection so that reproducible and comparable results could be obtained.

If saliva was allowed to stand at room temperature, 30 per cent destruction of mucin occurred in thirty minutes and 50 per cent in two hours, as estimated by the A.C.R.A. titer. If the sample was placed on ice immediately after collection, only slight destruction of mucin occurred within one hour of collection, although there was a 30 per cent reduction within two hours. In measuring the physical properties of saliva, therefore, all samples must be placed on ice and tests completed within one hour of collection if comparable results are to be obtained.

The mucin in the saliva is destroyed by mucinase, an enzyme normally present in saliva in the mouth, and also by an oxidation process in which ascorbic acid is involved.[8] Tests were carried out to inhibit mucinase activity and so possibly inhibit the changes in the physical properties of saliva. Eight enzyme poisons (sodium azide, potassium cyanide, sodium arsenite, sodium fluoride, mercuric chloride, potassium iodide, iodoacetic acid, and penicillin) were tested for their action upon salivary mucinase at room temperature. One-tenth milliliter of M/10 solutions of these agents was added to 2 ml. of saliva and the A.C.R.A. titer estimated every ten minutes for two hours and then half-hourly for an additional two hours. All these poisons were found to slow down destruction of the mucin, but none gave complete inhibition.

Collection of Sample.—The composition of saliva is not constant and is largely determined by the nature of the stimulation to the salivary gland. Therefore, in any comparison of samples of saliva it is necessary to collect the samples under conditions as near standard as possible. One popular "standard" method of collecting saliva is to collect it during the stimulation of chewing paraffin wax. Samples of saliva stimulated by chewing paraffin wax were, however, found to have a low mucin content, and readings could not be obtained for *spinnbarkeit* or flow elasticity, and extremely low readings were obtained for viscosity, tack, and A.C.R.A. titer.

"Resting" saliva was found to contain measurable amounts of mucin and was used for all further experiments. Resting saliva was collected with minimum stimulation by allowing the saliva to run naturally into the mouth and then voiding it into a test tube. Movement of tongue and cheeks was kept to a minimum.

The hour of collection of samples of saliva was found to be of great importance. Samples of resting saliva were collected hourly from four individuals from 9 A.M. to 6 P.M. for four days, the individuals' usual daily routine being otherwise unaltered. Great variation in A.C.R.A. titer, *spinnbarkeit*, flow elasticity, and viscosity occurred during the day. The variations were thought to be due to meals taken during the day, the effect lasting for different lengths of time.

These results showed very clearly the need for a standard method of collection and treatment of salivary samples, and in all further investigations the following standardized method was used: Samples of saliva as near resting as possible were collected between 9.30 A.M. and 11.30 A.M. when at least one and one-half hours had elapsed after any food or water had been taken. The saliva was allowed to run naturally into the mouth and then voided into a tube, the duration of collection being ten minutes. The volume was recorded and the sample placed on ice. All estimations were completed within one hour of collection. Samples were not collected from women during menstruation.

MEASUREMENTS OF PHYSICAL PROPERTIES

Using the standard method of collection and treatment of samples given here, it was possible to compare various samples of saliva. Day-to-day variation and effect of stimulation due to intake of various foods were studied.

In this investigation, measurements were carried out on the physical properties and mucin concentration of some 700 samples of saliva. These data were analyzed to see if any relationship existed in saliva between mucin concentration and the physical properties.

Day-to-Day Variation.—Samples of "resting" saliva were collected from the same individual every morning at 10 A.M. for two months to observe the day-to-day variation, and variations in all properties were observed. Table I demonstrates the range of these variations.

In a group of thirty young women, slight alteration was noted in the saliva during the winter months upon the first three days of the menstrual

TABLE I

DAY-TO-DAY VARIATION IN "RESTING" SALIVA OF ONE INDIVIDUAL

	MEAN OF DAILY READINGS	RANGE	MEAN DEVIATION	STANDARD DEVIATION	STANDARD ERROR
Volume (ml.)	1.95	1.5 - 3.4	±0.45	0.23	0.05
A.C.R.A. titer	6.5	4.5 - 7.5	±0.5	0.25	0.05
Spinnbarkeit (inches)	1.2	0.2 - 2.75	±0.75	0.9	0.2
Tack (Gm.)	5.3	4.8 - 5.8	±0.2	0.28	0.06
Viscosity at P_s (small ball at 30 degrees)	5.9	4.3 - 9.8	±1.4	0.58	0.14
Density	1.015	1.01- 1.02	±0.005	0.005	0.005
Tyrosine, Gm./100 ml.)	19.2	14.1 -29.0	±4.2	2.9	1.0
Surface tension (dynes/cm.)	18.8	15.2 -26.0	±0.38	7.8	1.75

TABLE II

VARIATION IN SALIVA DUE TO INGESTION OF FOODSTUFFS

	VOLUME (ML.)	A.C.R.A. TITER	*Spinnbarkeit* (INCHES)	TACK (GM.)	VISCOSITY (CENTIPOISES AT P_5)	FLOW ELASTICITY (MM.)	CHEMICAL ESTIMATION OF MG. TYROSINE/ 100 ML.
Resting saliva (control)	1.95	6.5	1.2	5.3	5.9	0	19.2
After ingestion of:							
milk	4.8	10	4	5.80	8.0	5	24.4
water (20° C.)	2.2	10	5.25	5.4	13.5	7	–
coffee	2.9	10	5	5.2	11.2	6	27.0
tea	5.2	9	6	5.65	9.4	6	–
orange	4.7	11	2.0	5.45	10.0	8	–
lemon	3.6	9	2.75	5.45	8.4	5	23.0
apple	3.9	10.5	6	5.55	16.2	10	–
biscuit	4.2	10	3.0	5.20	8.3	4	–
cheese	4.6	10	3.25	5.70	8.0	3	–
toffee	5.4	10	4.25	5.20	8.0	4	–
bread and butter	4.6	9	1.75	5.05	8.7	5	29.4
*Standard error for resting saliva from 20 readings	0.05	0.05	0.2	0.06	0.14	–	1.0

*The difference between the mean reading for any one test following stimulation with any particular food and that of the mean reading of the resting saliva is considered significant if it is numerically greater than twice the standard error for the test.

period. Higher readings were obtained in A.C.R.A. titer and *spinnbarkeit*. Further investigation of this preliminary finding is necessary.

Variation With Food.—Samples of saliva were collected under standard conditions. Then a sample of food was eaten and, five minutes after the completion of the food, samples of saliva were again collected.

A significant difference was noted in all properties, except tack, in the samples collected after ingestion of the various foodstuffs. Results are given in Table II.

Relationship of Properties.—Data obtained from 700 samples of saliva were analyzed. The mucin content, as measured by tyrosine content and A.C.R.A. titer, was plotted against viscosity, *spinnbarkeit*, and tack (Fig. 3). Some correlation was found to exist between A.C.R.A. titer and both viscosity and *spinnbarkeit*. No correlation between tyrosine and *spinnbarkeit* was found. The correlation coefficient between A.C.R.A. and *spinnbarkeit* was 0.81, between A.C.R.A. and viscosity 0.60, and between *spinnbarkeit* and viscosity 0.89. In all other cases it was below 0.15.

DISCUSSION

The physical properties of saliva are determined by the state of the mucin present and not on the actual concentration. It appears that the percentage of "polymerized" mucin (that is, unhydrolyzed mucin) determines the A.C.R.A. titer and the *spinnbarkeit* and viscosity values, these all being dependent on the presence of the whole complex mucin molecule. A highly polymerized

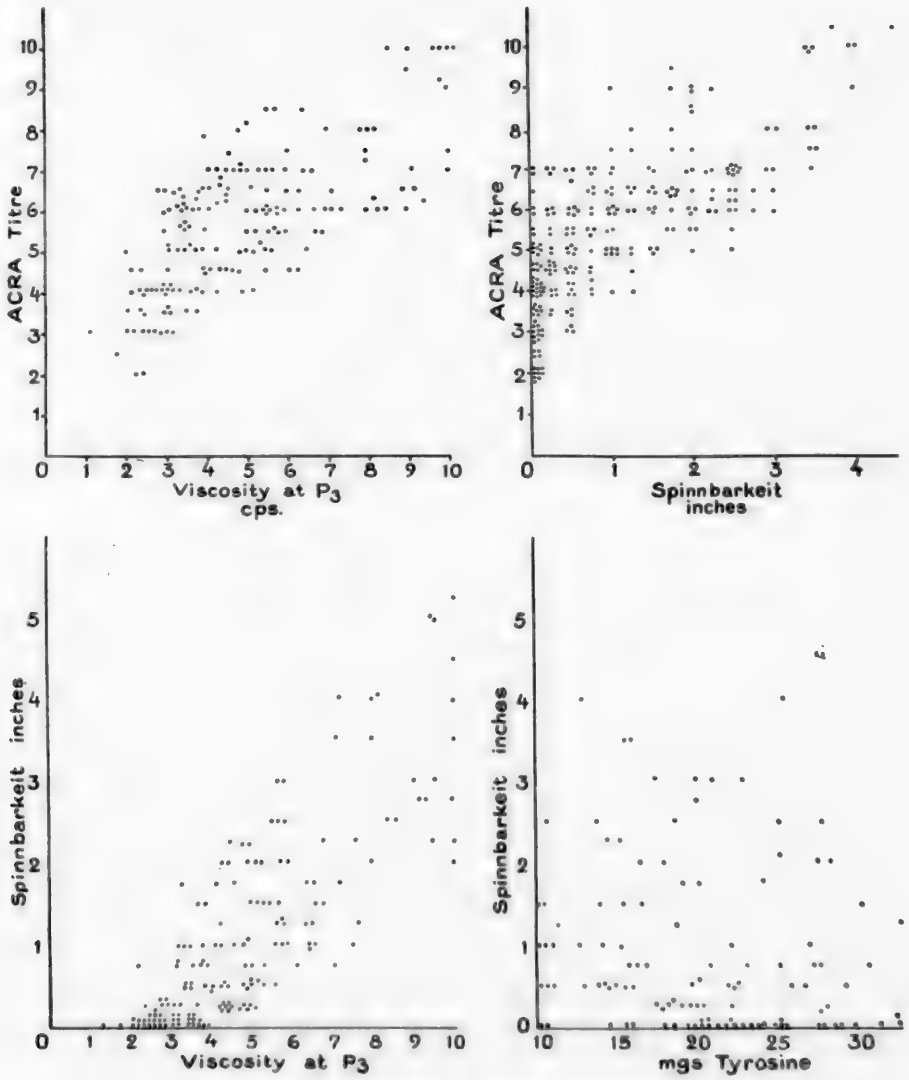

Fig. 3.—Correlation of salivary properties.

mucin gives high values in A.C.R.A. titer, *spinnbarkeit,* and viscosity. If depolymerized, the same mucin concentration will give low values in these properties. Evidence for this is found in the results obtained when saliva is incubated or allowed to stand at room temperature. The factors in saliva which influence the state of mucin are not known, but they are probably those known to affect the colloid state. Simmons[9] has shown that salt content can greatly influence viscosity of mucoid solutions, and pH has also been shown to affect the physical properties of saliva.

The effect of depolymerization on viscosity of mucoid solutions has been noted by Meyer and Palmer.[10] They studied the viscosity of mucoid of vitreous humor and found that it was not related to the concentration obtained on analysis of the mucoid. They stated that the loss of viscosity due to addition of acid to vitreous humor "is apparently not due to a change in the chemical make up of the molecule but to a partial breakdown of (the) long 'aggregates.' "

Because the physical properties of saliva are closely dependent on the state of the mucin present in the colloidal solution, and this is not stable, they vary greatly in value, depending on the conditions under which the samples of saliva are collected and treated. If any comparison is to be made between samples of saliva, it is obviously necessary to adhere to standard conditions of collection and treatment.

SUMMARY

Several physical properties characteristic of non-Newtonian fluids, such as anomalous viscosity, flow elasticity, tack, and *spinnbarkeit,* are described, and methods for measuring them are reported. Evaluations of methods for determining mucin content of saliva indicated that tyrosine estimation and a modified A.C.R.A. test gave satisfactory results on suitable samples.

Measurements on 700 samples of saliva made within one hour of secretion revealed some relationship between viscosity, *spinnbarkeit,* and the content of polymerized mucin as measured by the A.C.R.A. titer. No relation of these physical properties to tyrosine content or tack was found.

Variations in all properties were noted from time to time during the day and also from day to day in the same individuals. It appears that the methods of collecting and treating salivary samples are of great importance in studies of salivary properties.

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THE EFFECT OF A STANNOUS FLUORIDE-CONTAINING DENTIFRICE ON CARIES REDUCTION IN CHILDREN

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INTRODUCTION

INCREASED EFFORT is being made to resolve the problem of reducing the incidence of dental caries in children through the use of prophylactic dentifrice preparations.[1-6] While a lack of agreement exists in the reported efficiency of many of these preparations[1, 4, 7, 8] it is felt that the attention which has been focused upon the use of dentifrices may have had a beneficial effect by stimulating many persons to improve oral hygiene. It is difficult to imagine that thorough cleaning of the teeth at appropriate times would not partially alleviate dental caries incidence in children, especially in those who previously had failed to practice even moderate oral hygiene. A reduction in dental caries by the prompt brushing of the teeth with a conventional dentifrice immediately following meals has been reported.[9] Therefore, it is desirable to search for ways of improving toothbrushing habits in children, as well as providing effective dentifrices for reducing caries experience.

The incorporation of fluorides into a dentifrice as a means of increasing its value for reducing dental caries has long been considered an interesting possibility. This may be an expected consequence of previous work which clearly indicates a favorable reduction in dental caries in children using fluoridized water[10-12] or who participate in topical fluoride programs.[13-15] Furthermore, the choice of a dentifrice as the vehicle for an anticaries agent is ideal from the viewpoint of its availability to all classes of people. Milk, for example, was considered as a fluoride carrier, but this would mean the ingestion of widely different quantities of fluoride by different children. Further, there is the probability that fluorine in milk is less effective due to formation of an insoluble calcium fluoride.[2] Bread and salt have been considered but again their consumption by children is too variable to assure the ingestion of a proper amount of fluorine. Previous workers have used fluoride-containing dentifrices, but apparently with little success. A liquid and a paste dentifrice containing 500 ppm of fluorine as sodium fluoride were tested by Bibby[1] in both children and adults. No evidence of caries reduction was reported after a two-year study period. However, McClendon[3] reported a significant reduction in new cavities in adult students using a brown rock phosphate dentifrice. Shaner and Smith[2] reported that a sodium fluoride dentifrice con-

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taining 2500 ppm of fluorine as sodium fluoride used twice daily under supervision "can produce a reduction in the counts of acidogenic organisms in the saliva of humans."

It appears that if the proper dentifrice and fluoride combinations were used it might be possible to produce a significant reduction in dental caries experience in children. The nature of the abrasive seems to warrant careful consideration. Bibby[1] has stated that the fluoride dentifrice used in his study still retained its "available" fluoride. "The possibility that the mixing of sodium fluoride with other dentifrice constituents rendered it inactive is largely ruled out by preliminary test tube studies which showed that the dentifrice mixtures exerted a solubility reducing effect on powdered enamel." In light of these findings, it was considered important to select the specific fluoride and dentifrice formulation on the basis of extensive preliminary work. Stannous fluoride has been shown to be superior to sodium fluoride in reducing enamel solubility,[16-18] whole tooth solubility,[19] and caries in rats,[20-22] hamsters,[23] and human beings.[15, 24] Moreover, good evidence exists that tin fluoride is less toxic than sodium fluoride.[21, 22] The selection of a dentifrice abrasive was made on the basis of enamel solubility reduction tests on numerous abrasive-fluoride combinations. This led to the selection of heat-treated calcium phosphate as the most compatible abrasive. The remaining constituents of the tooth paste, such as binder, humectant, flavor, and detergent, were of the type sold for use in conventional tooth pastes and are commonly available. The experimental tooth paste, No. 2, contained 0.4 per cent stannous fluoride (0.1 per cent fluorine). The control tooth paste, No. 1, was the same as No. 2 in every respect except that it did not contain stannous fluoride. The two dentifrices were indistinguishable by color, flavor, consistency, or in any other way. Both were packaged in plain white containers with only the identifying numbers printed on them.*

EXPERIMENTAL METHOD

After approval of the proposed program by the dental society and school board of Bloomington, Indiana, in May, 1951, approximately 800 parents in that community were contacted in relation to the participation of their children in a dental caries program. Through the cooperation of the city school system the parents were contacted by mail and given a form letter explaining the nature of the program. From replies to this letter 514 children were selected at random to participate in the study program. Each participating child was given an initial appointment, at which time he received a thorough prophylaxis by a dental hygienist and a full mouth bite-wing radiographic examination. The child was immediately examined by a dentist with the aid of a mouth mirror and explorer. The dental caries examination was completed by charting the additional lesions observed on the radiographic films. Following the oral and x-ray examination the subject was assigned to group 1 or 2 on the basis of estimated caries expectancy.

*The composition of the dentifrice used in this study was: heat-treated orthophosphate, 42 per cent; detergent (nonsoap), 2 per cent; humectant, 25 per cent; water, 29.2 per cent; binder, 1.4 per cent; stannous fluoride, 0.4 per cent; and flavor.

Estimation of relative caries expectancy was made by multiplying a subject's DMF surfaces at the time of the initial examination by a factor corresponding to his dental age or number of permanent teeth. These factors were previously determined from another large group of grade school children[25] and represent the average ratios of expected caries to caries history at each dental age. The subject was then placed in one of nine classes based upon the level of his caries expectancy. Within this expectancy class he was assigned to group 1 or 2 so that a continuous balance was maintained. The end result of this matching procedure was that each group contained nearly the same number of subjects in each caries expectancy stratum. The average expectancy at the start of the test was 5.30 for group 1 and 5.22 for group 2. If only those subjects who completed the study are considered, then the average expectancy was 5.26 for group 1 and 5.29 for group 2. The corresponding average number of DMF teeth at the first examination for those who completed the study was 7.85 for group 1 and 7.63 for group 2.

After the child was assigned to one of the groups, he was given a supply of the corresponding dentifrice and a new toothbrush. The entire family of each subject was also supplied with the same dentifrice to minimize the possibility that subjects might use another dentifrice which would otherwise have been available. Six weeks later the patient returned for an oral hygiene evaluation. At this time the parents were asked if they had any complaints or suggestions relative to the program and the child's mouth was examined for irritation, stain, general oral hygiene, and other factors that might represent the nature of the oral hygiene being practiced. The parents were questioned concerning the child's brushing habits, with emphasis on frequency and time of day, and about their likes and dislikes of the dentifrice they were receiving. At this visit, the child was asked to brush his teeth in the same manner as performed at home. The time required to do this and the method of toothbrushing were recorded by a hygienist standing in such a location that the child did not know of her presence. Following this, the child was given another appointment to return six months from his initial visit. At this time the hygienist again performed a thorough prophylaxis, bite-wing x-rays were again made, and the clinical dental caries data recorded by the same dentist. The order in which the children reported for examination was in no way related to the type of dentifrice being used. Precautions were taken to prevent the examining dentist from knowing or guessing which dentifrice a child was using. The dentist examined a child only after a thorough prophylaxis so that telltale signs of treatment, if any, were removed. The examination card upon which the dental findings were recorded was completely masked except for the blank spaces to be filled in. This procedure was adopted to minimize the possibility for bias in the examination, to make the initial and subsequent examinations completely independent of each other, and to equally distribute between the two groups the effect of unavoidable changes in standards of judgment which occur from day to day or over longer periods.

RESULTS AND DISCUSSION

Table I shows the caries experience for each group expressed in terms of several indices on the basis of average increase in caries units per person. This expression is used rather than proportion of total teeth attacked because the groups were balanced on the basis of caries expectancies of the individuals. Table I shows a 53 per cent reduction in DMF teeth for the group using the stannous fluoride dentifrice when compared to the control group. When the DMF surfaces index is used the reduction for the stannous fluoride dentifrice group is 72 per cent. Both of these reductions are highly significant as indicated by the low probabilities. It is interesting that the results by the two indices DMFT and DMFS are somewhat different. This could be explained in two ways: (1) the DMF surface index is more sensitive to the degree of severity of caries attack per tooth; and (2) the DMF tooth index as ordinarily used counts only new decay in previously caries free teeth, while the DMF surface index counts new decay in previously caries-free surfaces of all teeth. This latter point is thought to be the main reason for the difference. If the DMF tooth index is used to apply only to those teeth which are excluded by the conventional methods of computation, the percentage reduction in the No. 2 group is 100 per cent. This method of using the DMF tooth index takes into account 52 per cent of the teeth which developed new caries during the test. The conventional use of the DMF tooth index takes into account the other 48 per cent of the teeth which developed new caries. A rough average of the percentage reductions by the two methods would give a reduction in total DMF teeth of 77 per cent or about the same as the reduction by total DMF surfaces.

The last three columns in the first part of Table I show the caries increments by DMF surfaces divided into three components: proximal surfaces,

TABLE I

AVERAGE INCREASE IN CARIES FOR SIX-MONTH PERIOD

	DMF TEETH	TOTAL DMF SURFACES	DMF PROXIMAL SURFACES	DMF OCCLUSAL SURFACES	DMF BUCCAL LINGUAL SURFACES
Group 1 (Control) n = 197	0.701	1.812	0.701	0.574	0.538
Group 2 (SnF_2) n = 223	0.327	0.516	−0.072*	0.426	0.161
% Reduction	53.35	71.52	100.0	25.78	70.07
t Value	3.00	5.53	5.94	1.24	3.13
Probability	.003	<.00001	<.00001	0.22	.003

Analysis of Variance for Surface Types

SOURCE	MEAN SQUARE	D.F.	F VALUE	PROBABILITY
Dentifrices	58.61	1	36.86	<.01
Surfaces	4.82	2	3.03	.05
D-S Interaction	10.44	2	6.57	<.02
Error	1.59	1254		

*Although this figure indicates a decrease in caries, it is recognized that carious lesions may undergo arrestment but not repair. For computation of per cent reduction this figure was taken as zero.

occlusal surfaces, and buccal and lingual surfaces. It is apparent that the stannous fluoride dentifrice protected the different surfaces in widely different degrees. This phenomenon is called dentifrice-surface (D-S) interaction. The Analysis of Variance in Table I is computed for different surface types, and for both dentifrices and supplies several items of information. The high F value for dentifrices indicates that the three surface types taken together show a highly significant reduction in caries for the No. 2 group. The difference in caries rates for the three surface types is significant at the 0.05 level even though all the surfaces in the No. 1 group show about the same rate of caries attack. The F value for D-S interaction indicates that the difference in protection which the three surface types exhibit is significant with a probability near the 0.01 level.

The most marked protection against caries occurred on the proximal surfaces of the teeth in children using the stannous fluoride tooth paste. The 100 per cent reduction figure which is very highly significant indicates the possibility of complete protection for proximal surfaces. Occlusal surfaces showed the lowest degree of protection. They were the only surfaces in which the difference between control and experimental groups was not significant. The buccal and lingual surfaces showed about the same degree of protection as the average for all the surfaces.

Table II shows the number of subjects, average caries increments and the percentage reduction in caries within the different age groups. Two interesting aspects of these data should be noted: (1) the consistency with which the stannous fluoride tooth paste appears to have protected all age groups and (2) the definite trend toward increased protection with increasing age. This trend was tested for significance by a specific test[26] for that purpose. The probability of getting a sequence that is farther removed from an expected sequence where there is no real trend is only 0.002. Both groups have an unusually high proportion of 7- and 8-year-old children for a grade school sample. However, it is felt that the only effect of this disproportion would be to bias the results on the conservative side since the caries reductions for these ages are almost the lowest in the group.

TABLE II

CARIES INCREMENTS AS AVERAGE DMF SURFACES BY AGE

AGE YR.	GROUP 1 CONTROL		GROUP 2 SnF_2		REDUCTION %
	N	MEAN	N	MEAN	
6	14	2.07	16	1.56	24.6
7	39	1.77	38	0.76	57.1
8	42	1.00	42	0.81	19.0
9	18	1.28	21	0.33	74.2
10	12	1.67	21	0.14	91.6
11	15	2.87	14	1.21	57.8
12	11	1.73	20	0.40	76.9
13	12	2.67	21	0.29	89.1
14	21	2.48	13	0.23	90.7
15 and 16	13	2.15	17	0.06	97.2

In view of the reported failure of a sodium fluoride dentifrice to reduce dental caries[1] a positive result with a stannous fluoride dentifrice might be expected only if the compatibility of the fluoride and the dentifrice were improved. On the other hand, several factors may have contributed to the marked reduction of caries in such a short period of time. Tin in combination with fluoride is superior to the sodium salt for topical applications.[15, 24] The daily application of stannous fluoride to the teeth may enhance its effectiveness. Topical studies with sodium fluoride indicate that a prophylaxis prior to treatment increases the effect. The stannous fluoride tooth paste had its most marked effect upon the proximal surfaces which are generally considered the most difficult surfaces to clean by brushing. This may indicate that stannous fluoride is retained by the plaque or debris in the interproximal area and its effect thereby enhanced. If this is true, any anticaries agent may be more effective on the proximal surfaces, because those areas which act as food repositories may serve the same purpose for an anticaries agent.

Some considerations relative to possible toxicity of such a product are necessary before any unrestricted recommendation by the profession can be made, even in the light of such pronounced anticaries effect as demonstrated by these data. The experimental dentifrice used contained 1,000 micrograms of fluorine and 3,200 micrograms of tin per gram of dentifrice and each tube contained a total of 150 mg. of fluorine. If a child brushed his teeth with 1 gram of the fluoride tooth paste and swallowed the entire amount he would ingest 1 mg. of fluorine. Although it is improbable that any child or adult would swallow all of the dentifrice, such a circumstance would provide only approximately the amount of fluoride as contained in one liter of communal water at a fluorine concentration of 1 ppm. People living in areas where the water is naturally fluoridated above the optimum level frequently ingest several times that amount of fluorine daily for their entire lives with no serious effect.

SUMMARY

A stannous fluoride-containing dentifrice at a fluorine concentration of 1,000 ppm and a tin concentration of 3,200 ppm results in a pronounced reduction in dental caries in 6- to 15-year-old children after using the dentifrice in an unsupervised manner for a period of six months. When compared to an identical control dentifrice not containing stannous fluoride, a 53 per cent reduction in DMF teeth and a 72 per cent reduction in DMF surfaces resulted. There appeared to be complete protection against interproximal lesions, while the reduction in buccal-lingual surfaces was 70 per cent and occlusal surfaces 26 per cent. All of the reductions are highly significant except the reduction in occlusal surfaces. The reduction of caries appears to be related to the subjects' ages, with the greatest protection occurring in the older age groups of the children. The effect of possible toxic effects through the continued use of a stannous fluoride dentifrice is discussed.

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DISTRIBUTION OF DENTAL CARIES IN CHILDREN

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INTRODUCTION

THE purpose of this study is to examine quantitatively the distribution of dental caries in children. Despite the ever-increasing volume of statistical analyses appearing in the dental literature, data have rarely been summarized into frequency distributions. Even when this has been done it has been incidental to other work, and the characteristics of the distributions themselves have been ignored. A knowledge of these might throw some light on the nature of dental decay and provide a basis for a more effective treatment of dental data.

STATISTICAL METHODS

In the following section an outline is given of the statistical technics used in this investigation. For purposes of illustration they are applied to the results obtained for children of 11 years of age. The distribution of the number of smooth surfaces affected by caries for these children is given in Table I. The mean count is 1.97 carious surfaces per child; the standard deviation, 2.55 carious surfaces.

TABLE I

DISTRIBUTION OF SMOOTH SURFACE COUNTS FOR CHILDREN 11 YEARS OF AGE

COUNT PER CHILD	NUMBER OF CHILDREN		
	OBSERVED	POISSON	NEGATIVE BINOMIAL
0	52	19.7	52.2
1	34	39.5	31.4
2	23	39.5	20.4
3	9	26.4	13.5
4	5	13.2	9.1
5	7	5.3	6.2
6	6	1.8	4.2
7	2	0.4	2.8
8	2	0.1	1.9
9	2		1.3
10	3		0.9
11	0		0.6
12	1		0.4
>12	0		0.5
Total	146	145.9	145.8
χ^2		108.0	3.2
Degrees of freedom		4	4
Probability		<0.001	0.27

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Distributions of this kind appear frequently in nature. They can often be approximated closely by one of three well-known probability distributions: the binomial, the Poisson, or the negative binomial, all of which presuppose an underlying "chance" mechanism. Since caries-producing attacks might be thought to occur randomly in time, these theoretical distributions are of considerable interest in connection with dental data.

The Binomial Distribution.—If a group of children were homogeneous in regard to susceptibility to dental caries and length of exposure, and if all surfaces were equally likely to show decay, the proportion of children having x defective surfaces would be given by

$$P(x) = \frac{N.'}{x.'\ (N - x).'} P^x(1 - P)^{N - x},$$

where P is the average proportion of defective surfaces and N the number of surfaces in each mouth. This clearly represents a simplification of most situations since both individuals and surfaces are likely to vary in susceptibility. In addition, it may be difficult, as in the example given, to specify the value of N.

Poisson Distribution.—When P is small and N large, the expression given previously may be approximately written as

$$P(x) = e^{-m} \frac{m^x}{x.'},$$

where m is the average count per child. Since the likelihood of caries on most surfaces is small, and the number of surfaces involved often relatively large, the Poisson law might be suitable for some dental data, particularly since a knowledge of N is not required. Varying susceptibility from surface to surface would not influence the probability pattern. The frequencies expected for a Poisson distribution with the same mean as the observed sample are shown in Table I. It will be seen that agreement between the observed and the expected frequency distributions is not satisfactory. This is probably due to variation in susceptibility from one individual to another.

The mean and the square of the standard deviation, that is the variance, of a Poisson distribution are equal. This provides a convenient test of whether the Poisson distribution would describe a set of counts such as the previous. For this set of data, the variance, 6.50, is considerably greater than the mean, 1.97.

The Negative Binomial Distribution.—This distribution may be used to describe data of the Poisson type for which the variance is greater than the mean, and related by the expression

$$s^2 = \frac{m^2}{k} + m,$$

where k is an index of the degree of homogeneity of the individuals involved. The probabilities of the event occurring x times in the sample are given by the

successive terms of the following infinite series developed by Greenwood and Yule[1]:

$$\left(1+\frac{m}{k}\right)^{-k}\left\{1+\frac{k}{\left(\frac{k}{m}+1\right)}+\frac{k\,(k+1)}{2.'\left(\frac{k}{m}+1\right)^{2}}+\ldots.\right\};$$

for example,

$$P(O)=\left(1+\frac{m}{k}\right)^{-k}.$$

The general signifiance of this distribution has been discussed by Newbold.[4] It may arise from the combination of a set of Poisson distributions with different values of m. For this reason it seems particularly suitable for the distribution of dental caries, since the probability of occurrence of these may vary from person to person.

To estimate k, the observed variance may be equated to $\left(\frac{m^2}{k}+m\right)$, or the proportion of zeros may be equated to $\left(1+\frac{m}{k}\right)^{-k}$. Both of these methods, when applied to the previous set of data, give values for k of 0.87. The frequencies expected for a negative binomial distribution with $k = 0.87$ and $m = 1.97$ are given in Table I. The agreement between observed and theoretical distribution is very good.

If some estimate of k is available, the value of m may be determined from the proportion of individuals free of decay by means of the relation:

$$P(O)=\left(1+\frac{m}{k}\right)^{-k}.$$

If the ratio of m to k is small, this is approximately equivalent to the corresponding Poisson expression:

$$P(O)=e^{-m}.$$

In this case, the data would be satisfactorily described by a Poisson distribution.

DATA

The sample examined consisted of public school children from fifty schools in Caledon, Albion, and Chinguacousy Townships of Peel County, Ontario. This district was chosen because it had a fairly homogeneous social economic structure. With the exception of three small towns, it was a rural area. A previous survey had shown that the level of dental care was low. This, it was hoped, would diminish the modification of the natural pattern of decay resulting from operative and surgical procedures. The distribution of children by age and sex is given in Table II.

In this study, the dental examinations were carried out by one of us (R. M. G.) using mouth mirrors and S.S.W. No. 3 explorers. Daylight was supplemented by a portable electric spotlight, where electricity was available.

The findings were dictated to a clerk, who recorded them on charts. All carious surfaces detectable with the explorer and mouth mirror on the smooth surfaces were recorded. Pits or fissures in which an explorer could be so firmly fixed that the hand could be taken away were also recorded as carious, regardless of appearance. The occlusal, mesial, or distal surfaces involved in multiple fillings or cavities were counted separately, since it was impossible to say on which surface the cavity originated.

TABLE II

AGE AND SEX DISTRIBUTION OF SAMPLE

AGE (YR.)	FEMALE	MALE	TOTAL
6	39	55	94
7	79	77	156
8	71	93	164
9	60	69	129
10	64	77	141
11	62	84	146
12	81	82	163
13	77	70	147
14	33	60	93
Total	566	667	1,233

THE DISTRIBUTION OF CARIES COUNTS

Frequency Distributions.—The total number of carious surfaces per child was first computed and tabulated. If a tooth was missing, it was assigned the same count as could be seen on its opposite number on the other side of the mouth. If both were missing, the teeth were assigned the conservative caries score of 1.* The resulting data were summarized into the frequency distributions which are given in Table III. The contours of these distributions are very irregular, and there is marked preference for certain numbers. At the ages 6, 7, and 8 years, in particular, there is a suggestion that the data are not homogeneous but are made up of two distinct sets of counts. This is clearly seen in Fig. 1, where the distribution for age 8 years is presented. For this reason, the counts of each of the individual children were divided into subcounts, one of the carious smooth surfaces and the other of the carious pitted surfaces. The following surfaces were considered pitted: the occlusal surfaces, the buccal surfaces of the lower first and second molars, the lingual surfaces of the upper first and second molars, and the lingual surfaces of the upper incisors.

New frequency distributions were then constructed for the separate counts, and these are given in Tables IV and V. For smooth surface counts, the distributions are fairly uniform in contour. The contours of the pitted surface distributions are very irregular, and there is still a strong preference for even numbers at all ages. These features are readily apparent in Fig. 1.

*While it is not thought that much bias was introduced by this rule, further studies of extracted teeth suggest that a missing anterior tooth be given the score of 2 smooth surfaces affected, and a missing posterior tooth should be given the score of 1 smooth and 1 pitted surface affected.

Application of Probability Distributions.—The means and variances for the smooth and the pitted surfaces are shown in Table VI. The variances are always greater than the corresponding means and, in the case of the smooth

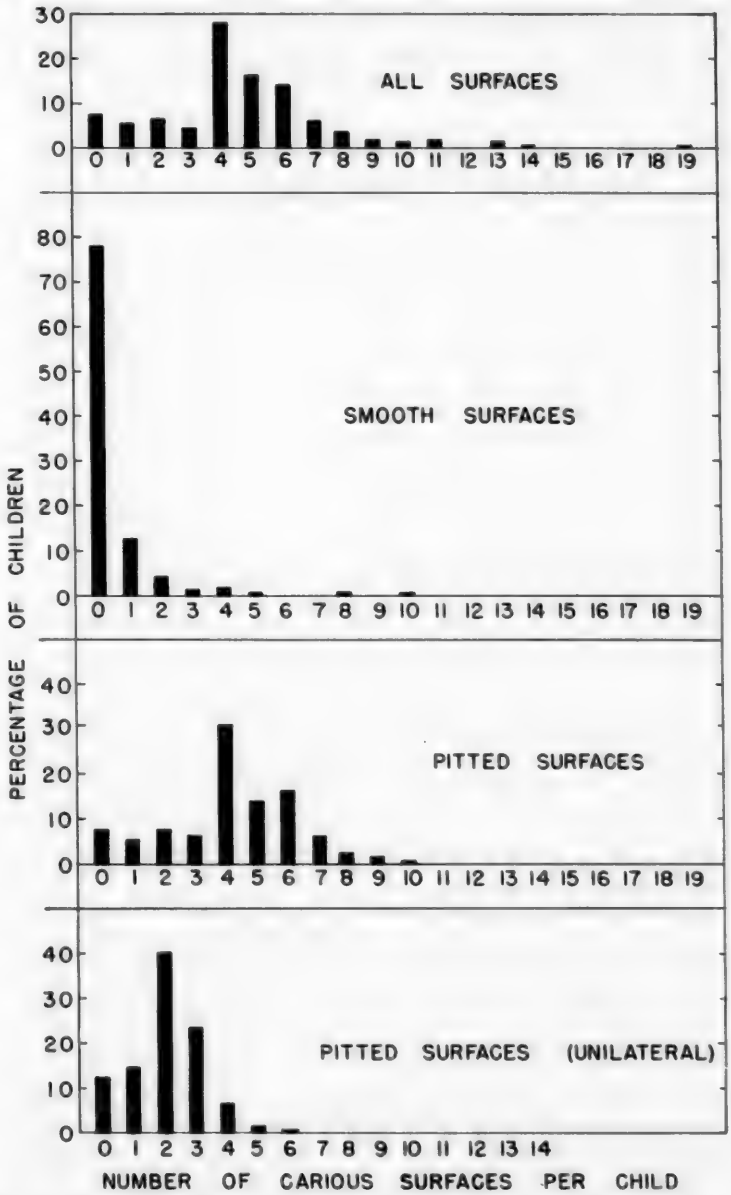

Fig. 1.—Distribution of number of carious surfaces for children 8 years of age.

surfaces, there is a suggestion of a nonlinear relationship between them. For these reasons, the negative binomial distributions were fitted to the observed smooth surface distributions. The resulting values for the constant k, and χ^2 are given in Table VII. None of the latter are near the critical values for the 5 per cent level of significance, indicating that the negative binomial fits the observed distributions satisfactorily. The constant k increases with the age of the children, reaching 1 at about age 13 years.

TABLE III

DISTRIBUTION OF TOTAL CARIOUS SURFACE COUNTS

COUNT PER CHILD	AGE (YR.)								
	6	7	8	9	10	11	12	13	14
	NUMBER OF CHILDREN								
0	21	19	13	10	5	3	6	2	1
1	18	17	9	6	3	3	4	1	-
2	13	13	11	7	12	2	4	5	-
3	9	14	8	11	7	12	4	1	2
4	18	46	46	23	16	12	8	8	2
5	6	27	27	15	14	15	15	6	3
6	6	8	23	22	15	17	12	5	4
7	-	7	10	12	19	8	10	8	2
8	2	2	5	9	15	17	14	14	8
9	-	3	3	3	7	6	11	9	7
10	-		2	6	7	5	15	9	5
11	1		3	1	4	11	8	11	6
12			-	2	4	6	8	10	9
13			2	1	5	6	8	11	8
14			1	-	1	6	3	8	5
15			-	-	3	5	4	6	3
16			-	-	-	3	4	5	4
17			-	-	2	-	4	3	4
18			-	-	-	2	4	4	4
19			1	-	-	1	5	4	2
20				-	-	1	1	6	2
21				1	-	1	1	2	2
22					-	-	-	-	2
23					-	2	5	-	1
24					-	1	-	1	1
25					-	-	1	-	1
26					-	1	1	1	1
27					-		1	3	1
28					-		-	1	-
29					-		2	-	-
30					-			-	-
31					1			-	-
32					1			-	1
33								-	-
34								2	-
35								-	-
36								-	1
37								-	1
.								.	
.								.	
.								.	
46								1	

Although "supervariation" is also present in the pitted surface counts, the fit of the negative binomial distribution is unsatisfactory, due to the irregularity of the contours. This irregularity takes the form of preference

TABLE IV

DISTRIBUTION OF SMOOTH SURFACE CAVITATION COUNTS

COUNT PER CHILD	AGE (YR.)								
	6	7	8	9	10	11	12	13	14
	NUMBER OF CHILDREN								
0	91	141	128	88	65	52	63	40	14
1	1	12	21	21	23	34	29	27	10
2	1	3	7	6	23	23	12	21	16
3	-		2	5	15	9	15	8	11
4	1		3	4	6	5	8	11	10
5			1	-	3	7	9	9	6
6			-	2	1	6	5	6	8
7			-	-	2	2	4	5	4
8			1	-	-	2	6	4	3
9			-	1	1	2	2	5	1
10			1		-	3	3	3	1
11					1	-	3	1	4
12					-	1	2	1	2
13					-		2	1	-
14					-			1	-
15					-			-	-
16					-			-	2
17					1			-	-
18								-	-
19								2	1
20								-	
21								1	
.								.	
.								.	
.								.	
33								1	

TABLE V

DISTRIBUTION OF PITTED SURFACE COUNTS

COUNT PER CHILD	AGE (YR.)								
	6	7	8	9	10	11	12	13	14
	NUMBER OF CHILDREN								
0	21	19	13	10	5	3	6	2	1
1	18	18	9	6	5	3	5	1	-
2	13	13	13	9	10	8	5	5	-
3	9	15	10	12	7	12	7	4	6
4	18	45	51	30	31	23	13	9	1
5	7	31	23	12	27	19	14	10	4
6	6	6	27	28	17	13	16	8	11
7	1	6	10	14	10	14	21	11	7
8	1	3	4	6	12	10	23	25	10
9		-	3	1	6	12	7	12	14
10		-	1	-	3	9	19	18	11
11				-	4	7	5	18	4
12				1	1	3	6	9	7
13					1	6	6	5	7
14					-	3	2	4	3
15					1	-	2	4	-
16					-	-	2	1	5
17					-	1	2	1	-
18					-		2		1
19					-		-		-
20					1		-		1

for certain numbers, and may be partially attributable to the correlation between the sides of the individual mouths (r varies from 0.68 to 0.80). To eliminate this source of irregularity, the scores for pitted surfaces were subdivided into individual scores for the left and right sides of the mouth. Distributions for the left side of the mouth are tabulated in Table VIII and summarized in Table VI. These are more regular in contour, as can be seen in Fig. 1, where the counts for age 8 years are given. Furthermore, the discrepancies between the variances and the means were diminished to the extent that the unilateral pitted surface distributions could sometimes be described by binomial distributions (Table IX). However, the observed zero frequencies are always higher than the expected. Much of the additional supervariation for the total pitted counts is probably due to the high correlation between counts for different sides of the mouth.

TABLE VI

MEANS AND VARIANCES FOR SMOOTH AND PITTED SURFACE COUNTS BY AGE

COUNT	STATISTIC	AGE (YR.) 6	7	8	9	10	11	12	13	14
Smooth	Mean	0.07	0.12	0.46	0.69	1.49	1.97	2.53	3.42	4.12
	Variance	0.22	0.14	1.65	1.98	5.24	6.50	10.71	21.89	15.30
Pitted	Mean	2.40	3.37	4.17	4.46	5.39	6.48	7.38	8.45	9.16
	Variance	4.29	4.05	4.55	5.23	8.92	11.67	14.66	11.67	13.53
Unilateral	Mean	1.20	1.65	2.04	2.21	2.70	3.23	3.67	4.24	4.61
pitted	Variance	1.24	1.12	1.35	1.35	2.36	3.35	3.75	3.28	3.31

TABLE VII

FIT OF NEGATIVE BINOMIAL DISTRIBUTION TO SMOOTH SURFACE COUNTS

STATISTIC	AGE (YR.) 6	7	8	9	10	11	12	13	14
k	0.30	0.46	0.20	0.36	0.62	0.87	0.67	0.70	1.23
χ^2	-	-	1.6	3.3	7.5	7.3	11.7	5.3	7.4
Degrees of freedom	-	-	2	2	4	7	9	9	9

TABLE VIII

DISTRIBUTIONS OF UNILATERAL PITTED SURFACE CAVITATION COUNTS (LEFT SIDES)

COUNT PER CHILD	AGE (YR.) 6	7	8	9	10	11	12	13	14
	NUMBER OF CHILDREN								
0	31	30	20	12	9	6	7	3	1
1	28	31	24	18	18	12	10	8	2
2	23	62	67	46	46	45	29	15	5
3	10	30	39	40	43	25	38	20	17
4	1	3	11	12	14	26	32	32	25
5	1		2	-	5	15	15	38	16
6			1	-	7	9	17	16	11
7				1	-	5	9	9	11
8					1	2	5	5	3
9					-	-	1	1	1
10					1	1			1

for non-mixed dentition

TABLE IX

FIT OF BINOMIAL DISTRIBUTIONS TO THE UNILATERAL PITTED SURFACE COUNTS (LEFT SIDES)

	AGE (YR.)								
	6	7	8	9	10	11	12	13	14
Estimated number of surfaces present	5	5	6	7	10	11	13	14	14
Estimated proportion of surfaces defective	0.24	0.33	0.34	0.32	0.27	0.29	0.28	0.30	0.33
χ^2	5.2	17.6*	14.7*	11.8*	9.5*	17.2*	15.8*	8.6	4.6
Degrees of freedom	2	3	3	4	4	4	5	5	5

*Statistically significant at 5 per cent level.

DISCUSSION

The data presented suggest that the negative binomial describes very satisfactorily the distribution of dental counts for smooth surfaces. This is in agreement with the idea that dental caries is a chance phenomenon with individuals differing in their susceptibility to tooth decay. An analysis of further data available gave a value of k for the theoretical distribution of about 1 for children over 12 years of age, as is suggested from the results of the previous study. With younger children, k increases with age during the time in which teeth are erupting and have not as yet manifested many cavities. In this period, the individuals may tend to be less alike than subsequently when all teeth are present.

The statistical treatment of pitted surface counts presents peculiar difficulties, mainly due to the marked irregularities of the distribution contours. The distributions for single sides of the mouth are more regular, but involve only a limited number of tooth surfaces, necessitating the use of some form of binomial distribution. That the underlying distribution is of a true binomial type is hardly to be expected because of the obvious presence of a number of variable factors, particularly the number of tooth surfaces involved. In addition, correlation between some of the pitted tooth surfaces both within the mouth and between individuals would tend to increase variability, while the differing susceptibility of the tooth surfaces would tend to decrease variability. This would be most evident during the early ages when the occlusal surfaces of the upper and lower first molars and, to a lesser extent, the buccal surface of the lower first molar are contributing the major portion of the caries.

It is possible, however, to develop a probability model which allows for variation in the proportion of affected pitted surfaces from individual to individual. The application of this to most ages does not seem worth while since the number of tooth surfaces may vary considerably. However, the process can be illustrated for the group of children 12 years of age, for which the number of surfaces is relatively constant. Assuming that the distribution of the proportion affected is given by the beta distribution,[3]

$$f(p) = (1.38 \times 10^{9})\ p^{7.9}\ (1-p)^{21.6},$$

the proportion of children with x carious surfaces can be obtained by integration over the range of p:

$$P(x) = \frac{13.!}{x.!\ (13 - x).!} \int_0^1 f(p)\ p^x(1 - p)^{13-x}dp.$$

The expected frequencies obtained from this are given in Table X, together with the corresponding values for the binomial distribution. Considerable improvement in fit is produced by this modification of the theoretical distribution. The underlying distribution of susceptibilities has a mean of 3.7 carious surfaces per child and a standard deviation of 1.0 carious surfaces. The corresponding values for smooth surfaces at this age are 2.5 carious surfaces and 3.1 carious surfaces. This supports the possibility, arising from the fit of the binomial at ages 13 and 14 years, that there may actually be less variation among individuals in terms of pitted surfaces than in terms of smooth.

The fact that in these data the proportion of pitted surfaces affected is relatively constant at all ages suggests that variation may arise during the process of the tooth formation, decay depending considerably on the morphology of the teeth at the time of eruption. The smooth surface counts may depend to a greater extent on the conditions affecting the tooth after eruption, since the proportion of smooth surfaces decayed increases manifold between 6 and 14 years.

TABLE X

DISTRIBUTION OF UNILATERAL PITTED COUNTS FOR CHILDREN 12 YEARS OF AGE

COUNT PER CHILD	NUMBER OF CHILDREN		
	OBSERVED	BINOMIAL	COMPOUND BINOMIAL
0	7	2.2	4.6
1	10	11.1	15.3
2	29	26.3	27.1
3	38	38.0	33.2
4	32	37.4	31.3
5	15	26.5	23.8
6	17	13.9	14.9
7	9	5.5	7.8
8	5	1.6	3.4
9	1	0.4	1.2
10		0.1	0.4
Total	163	163.0	163.0
χ^2		15.2	7.9
Degrees of freedom		5	6
Probability		0.01	0.25

By assuming the statistical distributions discussed previously, the proportion of the individuals who are free of caries might be used to estimate the mean number of carious surfaces per individual. Knutson[2] carried out a similar scheme with teeth as units, but did not provide any theoretical basis for the relationship used. For smooth surface caries, the observed proportion of children free of decay can be equated to the expected proportion of the negative binomial distribution. For children of a similar age, the appropriate value of k may be

substituted in the relation of P(O) to give an estimate of m. The value of k is not critical, and fairly good estimates may be obtained in the older ages by letting k equal 1. In this case,

$$m = \frac{\text{number of children showing decay}}{\text{number of children free of decay}}.$$

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TISSUE-DESTRUCTIVE PRODUCTS OF GINGIVAL BACTERIA FROM NONSPECIFIC GINGIVITIS

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PREVIOUS work[1] demonstrated the presence of tissue-destructive agents in cell-free extracts of gingival bacteria. Extracts of one of these tissue-destructive agents, which occurred somewhat irregularly, gave a histamine-like reaction on injection in rabbit skin. Another, which was consistently present in extracts prepared from inflamed gingiva, was a spreading factor (hyaluronidase). That such a spreading factor could play a part in producing gingivitis was supported by the demonstration that topical applications of hyaluronidase on human gingiva produced tissue changes similar to those found in gingivitis. The idea that hyaluronidase is important in gingival destruction is supported by histologic studies which show that the amorphous ground substance in the gingival connective tissue contains hyaluronic acid and that this is removed in local inflammatory areas.

Evidence of the existence of two tissue-destructive agents suggested that other such agents could be identified in bacteria-free preparations of gingival bacteria. That an enzyme acting on the chondroitin sulfate might be present is suggested by the observations[2-4] that chondroitin sulfate disappears in areas of gingival inflammation; the likelihood that a collagenase might be involved is suggested by the progressive destruction of collagen fibers in advanced gingivitis.[5]

Therefore, it was decided to prepare bacteria-free filtrates from bacterial deposits from the gingivae of individuals with gingivitis and normal gingiva, to test them for activity against hyaluronic acid, chondroitin sulfuric acid, and collagen, and also to look for further evidence of the presence of histamine and, if possible, to determine whether the occurrence of these different agents were in any way interrelated.

In addition, extracts of pure cultures of several microorganisms isolated from the human gingival pocket were tested for activity against the substrates listed. The work on gingival filtrates and bacterial cultures will be reported in separate sections.

A. ANALYSIS OF BACTERIA-FREE FILTRATES PREPARED FROM GINGIVAL DEPOSITS

1. *Hyaluronidase Activity.*—

Substrate: Potassium hyaluronate was employed as the substrate for the determination of hyaluronidase activity in the filtrates. The hyaluronate was

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prepared from human umbilical cords according to the method described by Byers, Tytell, and Logan.[6] After acetone treatment, the material was digested with trypsin and the hyaluronate precipitated from the cleared, dialyzed tryptic digest by ethanol saturated with potassium acetate. In our experience this method gives a good yield of the potassium hyaluronate of satisfactory purity.

Tests with bacterial filtrates: Twenty bacteria-free filtrates were prepared and tested for hyaluronidase activity. As much bacterial deposit as possible was collected from the gingival region and gingival pocket of a patient, using standard periodontal instruments. This material was suspended in 1 ml. of distilled water in a small test tube, shaken for thirty seconds in a dental amalgamator, and then filtered through a sintered glass filter. Tests for sterility of the filtrates were always carried out.

In testing the action of the filtrate against potassium hyaluronate, the mucin clot-prevention test described by Burnet[7] was employed. Serial two-fold dilutions of hyaluronic acid were prepared in small test tubes, and one drop from each test tube was dropped into a Petri dish filled with a shallow layer of acid alcohol. With increasing dilutions, the form of the drops become progressively more imperfect, and the end point is taken as the dilution which forms a temporary annulus which immediately disintegrates peripherally. Dilutions beyond this point give only a diffuse haze which dissipates rapidly.

After establishment of the end point, the gingival filtrates were added to standard hyaluronic acid solutions and incubated for one hour at 37° C., and clot formation was compared with that of a standard hyaluronidase control.

The activities of the gingival filtrates in preventing clot formation as compared with testicular hyaluronidase were given arbitrary values ranging from 3+ to 0, a 3+ activity corresponding to the activity of 150 TRU of a standard hyaluronidase solution. It is fully realized, however, that because of differences in the action of bacterial and testicular hyaluronidases, the same numerical designations do not indicate identity in effects.

Results: The results are summarized in Table I. Filtrates obtained from seven individuals with generalized chronic marginal gingivitis all were active against potassium hyaluronate. Activity was also noted in filtrates from two patients with several local gingival inflammatory areas. The gingival pocket depth in these areas was 3 mm. or more. Filtrates obtained from four individuals with a few scattered local inflammatory gingival areas and where the pocket depth was less than 3 mm. did not produce any effect on the substrate. Six filtrates obtained from individuals with clinically normal gingiva were inactive against potassium hyaluronate after one hour's incubation at 37° C.

One filtrate was obtained from an individual with a desquamative type of gingivitis. This filtrate was very active in terms of hyaluronidase activity. In this connection it is interesting to note that Engel, Ray, and Orban[8] found increased collagenase-like activity in the gingival tissues of patients with desquamative gingivitis. To three filtrates known to possess hyaluronidase activity, a hyaluronidase inhibitor (heparin) was added. This eliminated the action of the filtrate on potassium hyaluronate.

TABLE I

SUMMARY OF ANALYSES OF FORTY-THREE BACTERIA-FREE FILTRATES PREPARED FROM GINGIVAL DEPOSITS REMOVED FROM INDIVIDUALS WITH OR WITHOUT GINGIVAL INFLAMMATION

CLINICAL DIAGNOSIS	AZOCOLL											
	HYALURONIC ACID						COLLAGEN					
	TOTAL NUMBER OF FILTRATES TESTED	ACTIVITY					TOTAL NUMBER OF FILTRATES TESTED	ACTIVITY				
		NUMBER OF ACTIVE FILTRATES	DEGREE OF ACTIVITY					NUMBER OF ACTIVE FILTRATES	DEGREE OF ACTIVITY			
			3+	2+	1+	±			3+	2+	1+	±
Generalized chronic marginal gingivitis	7	7	2	3	2		2	2	1		1	
Local inflammatory areas, deep pockets	2	2		1	1		4	3		1	1	
Few local inflammatory areas, shallow pockets	4	0					4	3			2	1
Desquamative gingivitis	1	1	1									
Clinically normal gingiva	6	0					2	0				
	CHONDROITIN SULFATE						HISTAMINE					
Generalized chronic marginal gingivitis	1	0										
Local inflammatory areas, deep pockets	1	1			1		2*	0				
Few local inflammatory areas, shallow pockets	1	0					3	0				
Desquamative gingivitis												
Clinically normal gingiva	2	0										

*Filtrates prepared by extraction of biopsy material.

2. *Activity Against Chondroitin Sulfate.—*

Substrate: Chondroitin sulfate was prepared from dried nose cartilage of cows, according to the method of Meyer and Smyth[9] in which the powdered cartilage is extracted with 10 per cent $CaCl_2$ and precipitated with acid alcohol. The precipitate is redissolved, poured into 0.2N HCl, and Lloyd's reagent is added. The solution is then precipitated with alcohol, and extracted with water, and the clear, viscous solution obtained is poured into glacial acetic acid. A fibrous precipitate is obtained which is washed and dried to a powder. The average yield was 7 Gm. of powder from 40 Gm. lots of dried cartilage.

Tests with bacterial filtrates: The filtrates were prepared in the way previously described. Five gingival filtrates were tested using the mucin clot-prevention test already described, except that the incubation period was twenty-four hours at 37° C.

Results: The results are listed in Table I. Of the five gingival filtrates tested, one, prepared from gingival deposits from an individual with local inflammatory areas and deep gingival pockets, showed activity. The activity was marked but not very strong after an incubation period of twenty-four hours.

Incubation of chondroitin sulfate with 150 TRU of a testicular hyaluronidase preparation for twenty-four hours at 37° C. showed a considerably greater decrease of viscosity of the substrate than that produced by the filtrate.

3. *Collagenase* Activity.—*

Substrate: Hide powder was used as the source of collagen. The collagen was coupled with an azo dye according to the method described by Oakley, Warrock, and van Heyningen.[10] Azocoll, the brick-red product of the coupling reaction, is split by the action of collagenase with resulting release of the dye, which then gives a red color to a clear suspending fluid.

Tests with bacterial filtrates: Twelve filtrates were prepared in the way previously described. Azocoll was suspended in a small volume of distilled water in a test tube and the filtrate added. In this respect the method described by Oakley, Warrock, and van Heyningen,[10] which employed mannucol as the suspending agent, was modified. When sterilized mineral oil is layered above the azocoll suspension, the azocoll collects at the interface between the oil and the mixture and, if splitting occurs, the azocoll particles do not interfere with the color of the mixture.

As a control for collagenase activity, extracts of *Clostridium welchii* (Type A) grown in thioglycollate broth for forty-eight hours at 37° C. were prepared and standardized. The color intensity produced by the action of the standardized *Cl. welchii* filtrate after twenty-four hours' incubation at 37° C. with azocoll was given an arbitrary value of 3+. The color which developed by the action of the gingival filtrates on azocoll after twenty-four hours' incubation at 37° C. was read against this standard.

Cl. welchii (Type A) also produces other enzymes in addition to collagenase, among them a hyaluronidase. For control purposes the actions of testicular hyaluronidase and bacterial hyaluronidase, as well as those of pepsin and trypsin, were tested on azocoll.

*The term "collagenase" is used here to designate any protease which can catalyze the breakdown of azocoll, and specificity is implied.

Results: The results are listed in Table I. Filtrates obtained from patients with clinically normal gingiva were not active against azocoll, whereas most filtrates (eight of ten) obtained from patients with various degrees of gingivitis were active against the substrate. It is of some interest to note that in the clinical group ("few local inflammatory areas with shallow gingival pockets"), three of four filtrates obtained split the azocoll.

That the collagenase is independent of the hyaluronidase activity is shown by the fact that a corresponding group of four filtrates tested for hyaluronidase activity did not produce any effect on hyaluronic acid.

4. *Test for the Presence of Histamine.—*

Method: The method used was described by Rosenthal and Tabor.[11] It is based on the formation of a characteristically colored azocompound by histamine at a certain pH. Trichloroacetic acid was added to a test tube containing the collected gingival deposits. After fifteen minutes the extract was filtered off and the diazo reagent added. The solution was alkalinized (pH 10.5), and methyl isobutyl ketone was added. After shaking and separation, the ketone was pipetted into test tubes containing 5 c.c. of barbital buffer at a pH of 7.7. After shaking and standing, the color of the ketone is compared to that of a series of standards containing a known amount of histamine. The method can detect 0.5 to 1 γ of histamine base in pure solution, and the color developed is specific for histamine.

Filtrates and tissue samples: Bacterial filtrates were prepared as for the other tests. In addition, tissue samples were tested. After the tissues removed by biopsy had been ground with mortar and pestle under trichloroacetic acid, the product was treated in the same way as the gingival deposits.

Results: Histamine was not detected in three extracts of gingival deposits or in two extracts of inflamed gingival tissues.

B. ACTIVITIES OF FILTRATES OBTAINED BY EXTRACTION OF PURE CULTURES OF SOME GINGIVAL MICROORGANISMS

1. *Hyaluronidase Activity.—*

Substrate: The substrate employed was potassium hyaluronate prepared in the way previously described.

Tests with bacterial filtrates: Six strains of β-hemolytic streptococci, five strains of *Streptococcus mitis,* and three strains of diphtheroid organisms isolated from the gingival pocket in patients with gingivitis were grown in 50 ml. of brain-heart infusion broth for twenty-four hours at 37° C. and filtered through sintered glass. Portions of 1 ml. were tested for hyaluronidase activity using the mucin clot-prevention test previously described.

Results: Of the six strains of β-hemolytic streptococci, two produced activity against potassium hyaluronate. In one case the activity was of the order of 3+, whereas the second strain produced a 1+ activity.

Of the five strains of *Str. mitis* tested, three produced activity against potassium hyaluronate. Two of the three strains produced a 3+ activity while

the third produced a 2+ activity. All three strains of the diphtheroid organisms tested produced hyaluronidase activity of the order of 1+.

2. *Activity Against Chondroitin Sulfate.*—

Substrate: The substrate chondroitin sulfate was prepared according to the method previously described.

Tests with bacterial filtrates: Three filtrates were prepared in the same way as before from cultures of β-hemolytic streptococci and one from a culture of *Str. mitis* from gingivitis. Activity against chondroitin sulfate was tested in the same way as for the gingival filtrates.

Results: None of the filtrates tested produced any detectable effect on chondroitin sulfate after twenty-four hours' incubation at 37° C. with the methods used.

3. *Collagenase Activity.*—

Substrate: The same azocoll preparation as was used previously was employed as a substrate.

Tests with bacterial filtrates: Two filtrates were obtained from broth cultures of β-hemolytic streptococci and three from *Str. mitis.* All microorganisms were isolated from the gingival pocket from patients with gingivitis. The activity was tested on azocoll in the same way as for the gingival filtrates.

Results: The two β-hemolytic streptococcal strains were not active against azocoll after twenty-four hours' incubation at 37° C., whereas all three *Str. mitis* strains produced activities of the order of 2+ against the substrate.

DISCUSSION

A consideration of the time of action of the various enzymes on the substrates is of interest not only because it demonstrates the rapidity by which the enzyme-substrate reaction takes place, but also because it casts light on their possible significance in destroying tissue elements in gingivitis. Hyaluronidase activity was always rapid, an incubation period of one hour at 37° C. being sufficient for determination of activity. Collagenase activity was considerably slower, the full color development from the splitting of azocoll requiring an incubation time of twenty-four hours. This suggests that hyaluronidase from gingival bacteria may play a more important part than the collagenase.

In respect to the collagenase, it is revealing to note that collagenase from *Cl. welchii* released color after two or three hours, from *Str. mitis* in eight hours, and from gingival bacteria nine to eleven hours. Evidence may be found in this that *Str. mitis,* which is prominent among the gingival bacteria, is a source of collagenase in the gingival area.

The action on chondroitin sulfate was not detectable until after twenty-four hours of incubation at 37° C. Since it is known[12] that bacterial hyaluronidase does not attack this substrate, and we showed that testicular hyaluronidase degraded chondroitin sulfate in eighteen hours, it is indicated that the breakdown of chondroitin sulfate is the result of a specific enzyme.

Table I reveals that at least three independently operating principles potentially capable of attacking various elements of the gingival tissues may be

identified in bacteria-free extracts of gingival deposits removed from individuals with varying degrees of gingival inflammation.

That they are independent of each other is illustrated by several observations. Thus some filtrates possessed collagenase activity but did not degrade hyaluronic acid to any measurable extent. When hyaluronidase inhibitor was added to three samples known to exhibit hyaluronidase and collagenase activity, the hyaluronidase activity was eliminated without interference with the collagenase activity. It was also observed that whereas testicular hyaluronidase after twenty-four hours' incubation at 37° C. showed a slight effect on azocoll (+), one filtrate with hyaluronidase activity, but no collagenase activity, did not attack azocoll after twenty-four hours of incubation.

In one filtrate prepared from a patient with generalized marginal gingivitis, all three enzymes were found to be present at the same time.

From Table I it will be observed that none of the enzymes under investigation could be detected in filtrates prepared from gingival deposits collected from patients with clinically normal gingiva. This does not necessarily indicate complete absence of the enzymes, but it does suggest that the amounts present are small compared to the amounts in filtrates obtained from patients with inflamed gingiva.

It has been demonstrated[13] that there is a large increase in the total number of bacteria present on the gingival tissues in gingivitis as compared to clinically normal conditions. This increased number of microorganisms could account for the existence of increased amounts of enzymes. On the other hand, an adaptive mechanism may also operate in the presence of a given substrate, allowing for an increased enzyme production by some bacteria. Thus, to take an example, the increase in number of *Str. mitis* as well as the increase in enzyme production by *Str. mitis* could account for increased amounts of hyaluronidase in filtrate preparations.

The presence in gingival pockets of enzymes capable of attacking elements of the gingival tissues seems to be of some importance with regard to the initiation of gingivitis. Their common occurrence makes it seem logical to consider such enzymes together with various forms of trauma or local irritations as exogenous predisposing factors in the development of gingivitis.

In brief, our findings emphasize the importance of bacteria and their products in the causation of gingivitis.

SUMMARY

1. The presence of agents capable of attacking hyaluronic acid, chondroitin sulfate, and a collagen preparation have been demonstrated in bacteria-free filtrates prepared from gingival deposits obtained from individuals with various degrees of gingival inflammation.

2. The active substances were not found in filtrates prepared from gingival deposits removed from individuals with clinically normal gingiva.

3. Histamine could not be detected in extracts of gingival deposits from patients with gingivitis or in extracts of inflamed gingival tissue.

4. Several microorganisms isolated from gingival pockets of individuals with gingivitis were shown to be capable of producing hyaluronidase and collagenase.

5. A discussion about a possible role of bacteria in gingivitis is given.

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NITROGEN CONTENT OF DENTAL PULP

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INTRODUCTION

POSSIBLY because of its small size, the individual dental pulp has not been the subject of extensive quantitative physiologic study. Pincus[1] reported his observations relating to tissue respiration and certain aspects of chemical composition as determined from pooled pulps of freshly extracted human teeth. However, the use of pooled pulps obliterates individual peculiarities that might exist and that might be of significance from a normal physiologic or pathologic standpoint. Furthermore, gross methods necessitating pooled samples impose a sometimes difficult requirement of sufficient individual specimens to permit even a minimum of such studies. It is highly desirable, therefore, to develop or adapt micro and ultramicro methods to the restrictions inherent in the pulp itself.

If respiratory activity of the individual dental pulp is to be studied, micromanometric procedures are necessary. However, the total volume of tissue required in these procedures[2] is sufficiently small that difficulty is encountered in routine gravimetric determinations of the mass. Nevertheless, an accurate measure of mass, or its equivalent, is imperative as a reference unit to which respiratory phenomena can be related. Total nitrogen has been used as such a measure and also has been recommended as a standard of reference to which oxygen consumption can be related.[3, 4]

Pincus[1] reported the nitrogen content of human tooth pulp as 10.1 per cent of the dry weight. This value presumably was determined by the macro-Kjeldahl method. It was decided, therefore, to investigate this problem further by the same method and then to attempt similar determinations by the micro method with the hope that ultramicro methods would become feasible eventually. Because various types of human dental pulp are not always available for analytic purposes, it was considered advisable to investigate also the possible effects on nitrogen determination that might result from preservation of this tissue in 10 per cent aqueous formalin solution.

MATERIALS AND METHODS

The tissues investigated were the pulps of bovine and human teeth. The bovine material came from animals between 2 and 3 years of age. The molars

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were extracted and placed in cold Ringer's solution as soon as killing and decapitation had been accomplished. The teeth were then taken to the laboratory, where they were cracked open and the pulps removed. The pulps were carefully examined under a stereoscopic microscope in order to remove all fragments of enamel and dentin. Some of the pulps were preserved in a 10 per cent aqueous formalin solution, and others were dried immediately, as indicated in Tables I and II. The pulps were cut into small pieces of approximately equal size to facilitate even dehydration and were placed in a constant-temperature drying oven. The samples of Groups A, B, C, and D of Table I and Group A of Table II were dried to a constant weight at temperatures between 95° C. and 100° C. The samples in the remaining groups were dried to a constant weight at 56° C. The longer drying at the lower temperature is recommended by the Association of Official Agricultural Chemists.[5]

TABLE I

NITROGEN CONTENT OF FRESH AND FORMALINIZED BOVINE PULP TISSUE DETERMINED BY VARIOUS METHODS

	TISSUE	METHOD OF ANALYSIS	NO. OF DETERMINATIONS	RANGE OF SAMPLE (MG. DRY WEIGHT)	MEAN (MG. N PER MG. DRY WEIGHT)	RANGE (MG. N PER MG. DRY WEIGHT)	STANDARD DEVIATION	STANDARD DEVIATION OF MEAN
A	Fresh	Macro-Kjeldahl	14	150-407	0.1257	0.1225-0.1284	0.0014	0.0004
B	Formalin	Macro-Kjeldahl	11	111-407	0.1256	0.1231-0.1286	0.0016	0.0005
C	Fresh	Nesslerization	122	1.3-2.6	0.120	0.08-0.15	0.013	0.001
D	Formalin	Nesslerization	75	1.3-2.6	0.117	0.08-0.15	0.015	0.002
E	Fresh	Micro-Kjeldahl	25	20-29	0.1273	0.1198-0.1336	0.0054	0.0011
F	Formalin	Micro-Kjeldahl	25	20-29	0.1249	0.1177-0.1437	0.0072	0.0014

TABLE II

NITROGEN CONTENT OF BOVINE AND HUMAN DENTAL PULP AS DETERMINED BY KJELDAHL METHODS

	TISSUE	METHOD OF ANALYSIS	NO. OF DETERMINATIONS	RANGE OF SAMPLE (MG. DRY WEIGHT)	MEAN (MG. N PER MG. DRY WEIGHT)	RANGE (MG. N PER MG. DRY WEIGHT)	STANDARD DEVIATION	STANDARD DEVIATION OF MEAN
A	Bovine fresh and formalin	Macro-Kjeldahl	25	111-407	0.1256	0.1225-0.1286	0.0013	0.0002
B	Bovine fresh and formalin	Micro-Kjeldahl	50	20-29	0.1261	0.1177-0.1437	0.0065	0.0009
C	Bovine formalin	Micro-Kjeldahl	5	2.24-3.10	0.1232	0.120-0.127	0.0082	0.0041
D	Human infant	Micro-Kjeldahl	18	13.8-20.8	0.1051	0.1001-0.1084	0.0025	0.0006
E	Human adult	Micro-Kjeldahl	9	2.5-15	0.1050	0.1000-0.1140	0.0040	0.0014

In accordance with the varying limitations of each analytic method, the sample sizes were of different magnitudes. In some cases, as in Groups A and B of Table I, 1 to 3 bovine molar pulps were required to provide a single sample. In other instances a single pulp would furnish enough tissue for 1 to 3 samples. In Groups C, D, E, and F of Table I and Groups B and C of Table II a single pulp was the source of from 1 to 10 samples.

The human material was of two types. The first type was from 6 infant heads obtained at autopsy (3 infants were born at full term and died shortly after birth, 3 were stillborn after eight months of gestation) and preserved in 10 per cent formalin solution from two months to four years. The tooth germs were dissected from the jaws, and the pulps were removed with the aid of a stereoscopic microscope. Each sample in Group D, Table II, required the pooling of from 4 to 9 pulps.

The second type consisted of the pulps of freshly extracted, noncarious teeth which were placed in 10 per cent formalin solution immediately after extraction. Twenty-two patients whose ages ranged from 14 to 48 years contributed 35 teeth. The majority of the teeth were removed because of the necessities of orthodontic or periodontic treatment. Within several hours of extraction, the teeth were cracked open and the pulps removed and dried as previously described. The number of pulps required to make up a single sample varied from 1 to 17.

The dry weight of most of the samples was determined on a Christian Becker Chainomatic analytical balance with a sensitivity of 0.1 mg. The dry weight measurements of human and bovine samples smaller than 15 mg., which were analyzed by the micro-Kjeldahl method, were determined on an Ainsworth keyboard-operated weight-carrier microbalance with a sensitivity of 2.5 μg.

Freshly prepared conductivity water as described by Fales and Kenny[6] was employed in the preparation of all solutions used.

Macro-Kjeldahl determinations were made according to the method described by Campbell,[7] with the exceptions that boric acid was used to collect the distilled ammonia[8] and bromcresol green-methyl red indicator was used.[9] By employing the boric acid solution in place of a standard acid there is one less solution to standardize and thus one less chance for error. The bromcresol green-methyl red indicator gives a very distinct color change from blue to colorless.

The nesslerization method described by Hawk and Bergeim[10] was employed in the analyses of Groups C and D of Table I. The colorimetric determinations were made on a Klett-Summerson photoelectric colorimeter.

The micro-Kjeldahl method used was that described by Beatty[11] with the addition of 30 per cent hydrogen peroxide to the digestion mixture as suggested by Hawk and Bergeim.[10] When analyzing samples of 2.2 to 3.5 mg. of dry weight, proportionately smaller quantities of reagents were employed. The apparatus employed was essentially that described by Keys[12] except that a vacuum was not employed.

Before any method of analysis was employed in the collection of data in this series, it was tried experimentally until confidence in its use and proficiency in its technics were attained. The accuracy of each method was checked by determining the nitrogen content of measured quantities of known solutions of reagent grade ammonium sulfate.

RESULTS

The observations reported were derived from a total of 304 determinations of total nitrogen by the several methods referred to previously and as indicated in Tables I and II. When fresh bovine dental pulp and that preserved in 10 per cent formalin solution were analyzed by the macro-Kjeldahl, micro-Kjeldahl, and nesslerization methods, there was substantial agreement in the nitrogen yields of each type of tissue (Table I). While the mean nitrogen values for formalin-preserved tissue appear slightly lower than for those of fresh tissue, this difference is not statistically significant.

When comparing mean nitrogen content as determined by the various analytic methods, it may be observed from Table I that the lowest mean yield is obtained in using the nesslerization technic. Since the macro-Kjeldahl method is presumed to be productive of the most accurate results, it served as a guide in evaluation of the other methods. Many laboratory workers are of the opinion that the lower values obtained by nesslerization are characteristic of the method. When this quality became clearly evident, the method was abandoned.

Inspection of Tables I and II shows close correspondence between the mean nitrogen values obtained by the macro- and micro-Kjeldahl methods. As might be expected, the range of individual values is greater when working on the micro level. The mean total nitrogen of bovine dental pulp, computed from all data derived from both macro- and micro-Kjeldahl analyses, was observed to be 0.1258 mg. per milligram of dried tissue (Table III).

TABLE III

COMPARISON OF MEAN NITROGEN CONTENT OF BOVINE AND HUMAN DENTAL PULPS DETERMINED BY KJELDAHL METHODS

	TISSUE	NO. OF DETERMINATIONS	RANGE OF SAMPLE (MG. DRY WEIGHT)	MEAN (MG. N PER MG. DRY WEIGHT)	RANGE (MG. N PER MG. DRY WEIGHT)	STANDARD DEVIATION	STANDARD DEVIATION OF MEAN
A	Bovine fresh and formalin	80	2.2-407	0.1258	0.1177-0.1437	0.0037	0.00042
B	Human formalin	27	2.5-20.8	0.1050	0.1000-0.1140	0.0031	0.0006

Human dental pulp preserved in 10 per cent formalin solution was analyzed by the micro-Kjeldahl method only (Table II). The mean total nitrogen values of infant and more mature tissue were observed to be almost identical. The mean nitrogen content of human dental pulp was found to be 0.1050 mg. per milligram of dried tissue (Table III).

DISCUSSION

A comparison of mean total nitrogen of bovine and human pulp tissue (Table III) reveals a significant difference. Since the series of human material shows no age differences, and the macro- and micro-Kjeldahl methods compare very favorably as to reliability, it is suggested that this difference is an expression of species peculiarity.

A critical comparison of mean nitrogen content of human dental pulp observed in this investigation with that reported by Pincus[1] is difficult because he does not elaborate on his methods in this regard. But since he does report that "nitrogen (Kjeldahl) was 10.1 per cent on a moisture and ash free basis . . .," the results of this study suggest that the nitrogen value he obtained is too low.

SUMMARY

1. The mean total nitrogen (Kjeldahl) of human dental pulp was observed to be 0.1050 mg. per milligram of dried tissue and that of bovine pulp 0.1258 mg. It is suggested that this difference is a species peculiarity.

2. Age of the individual does not appear to influence the nitrogen content of human dental pulp.

3. Preservation of dental pulp in 10 per cent aqueous formalin solution does not significantly alter its suitability for nitrogen analysis by the Kjeldahl method.

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Compiled by Hamilton B. G. Robinson,* Editor, College of Dentistry, Ohio State University, Columbus, Ohio

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I. Inaugural Address: The International Aspects of Our Association With Special Emphasis on Japan

Geo. C. Paffenbarger

Senior Research Associate of the American Dental Association at the National Bureau of Standards

I take a special interest in the international aspects of our Association. It is particularly pleasing that at this meeting two new sections of the Association have been accepted; one in Germany, the other in Japan. I believe that one of the primary purposes of our society is to create and to promote international good will as well as to do what our constitution states we should do; that is, "(b) to encourage and to facilitate cooperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology."

There are so many things that seem to create international ill will that all of us must make every effort to conduct ourselves in such a way that we will create international friendships. We can do this, of course, in a number of ways but one of the most successful is the international exchange of persons. Since the termination of the war this is being carried out extensively. Some of us have been sent to other countries and we have all had foreign visitors in our laboratories. You notice I said "in our laboratories." I wish I could say also in our homes. It is in our homes that we will get to know and to appreciate them and they us. When I traveled in other lands I considered an invitation to a person's home as the ultimate in courtesy and friendship, and I am sure that visitors to the United States feel the same way.

Today I want to take a few minutes to discuss a specific trip that it was my good fortune to make.

In 1950, at the suggestion of the Japan Dental Association, the Supreme Command for the Allied Powers (SCAP) invited the American Dental Association to send a five-man mission[1] to Japan for a period of thirty days. The

*The editor was assisted in the preparation of these proceedings by the secretary-treasurer, Dr. E. H. Hatton, and by the assistant secretary-treasurer, Dr. Dan Y. Burrill.

purpose of this mission was to assist the Japanese in all phases of dentistry. I was fortunate in having been selected as a member of that mission.[2] As a result I was in Japan all during July, 1951. I am, therefore, what you would consider a thirty-day expert on Japan with a three years' cooling-off period.

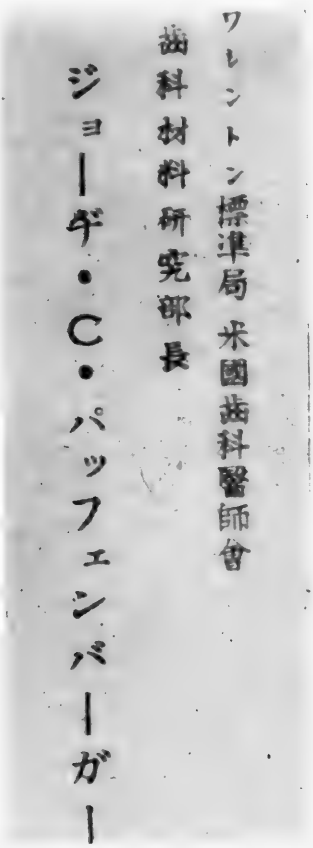

Fig. 1.—Example of personal card made up for each member of the Dental Mission by the Japan Dental Association. Translation is George C. Paffenbarger (left-hand column), Dental Material Research Chief (middle column), Washington, National Bureau of Standards, American Dental Association, (right hand column).

The dental mission spent half of July traveling in Japan, from Tokyo west to Gifu where we went fishing with cormorants, to Nagoya to Kyoto, the old capital of Japan and a cultural and art center. Here are china factories, cloisonne handiwork, the damascene workshops, the craftsmen who create the fans, the lanterns and the wood block prints which we all admire. This beautiful city of Kyoto was not bombed during the war, just as the shrine cities of Nikko and Nara and the Emperor's palace grounds were not bombed. Why were these places spared? Because they had little military importance and for psychological reasons. Then on to Osaka, the great manufacturing center which took a fearful pounding during the war. Next was Hiroshima, the city utterly wiped out by the atomic bomb. Why was Hiroshima chosen

for this first destructive blast? I am told that it was one of the principal embarkation points for the Japanese Army and that the terrain was such that maximum effects would occur.

From Hiroshima we journeyed to Kokura on the southern island of Kyushu and to Beppu, the hot-spring resort city—then back to Tokyo, 600 miles away.

From Tokyo we took another trip east to Sendai, one of the principal cities of eastern Japan, then down to Nikko, the beautiful shrine city tucked in the mountains, then back to Tokyo. It is of interest to note that in Sendai, which has a population of about 350,000, nearly 10 per cent are Christians whereas the over-all average in Japan is somewhere between 1 and 2 per cent.

Everywhere we went, lecture meetings were held with the local and state (prefectural) dental societies, and conferences with and visits to dental educational institutions, research and health centers, hospital and social agencies.

Fig. 2.—Japanese flower-bearers at Gifu.

There are 80 some million Japanese on these islands. I probably conversed with 150 English-speaking Japanese dentists. With the other dentists (about 26,000), I could neither converse nor even exchange personal cards with any degree of satisfaction. I could not read their cards and they could not read mine. The Japan Dental Association solved this difficulty by having our personal cards printed in Japanese (Fig. 1). I want to impress upon you that giving out these cards was a hazardous business. On more than one occasion I found myself about to give out some Japanese dentist's card which had just been handed to me a short time before. I solved this difficulty by keeping my cards in one pocket and all of the other cards that were handed to me in another pocket. Even then I had some trouble as it became a bit complex with handing out cards, receiving cards, shaking hands, bowing, and talking to dozens of official greeters who were always present when we stepped off the train.

I know that my original family name (Pfaffenberger) literally means priest-on-the-mountain, but I was in for a rude awakening when Paffenbarger

was transposed into Japanese characters and then translated literally back into English. There was no trouble with George or the Paf-fen part of the name. The trouble was on the bar-ger end. Please note the next to the last character from the bottom (Fig. 1)—this is the Japanese designation for a horse and is pronounced "bä." In fact, the character does look something like a horse with the front and hind legs and the two ears. Then comes a dash followed by the final Japanese character, that for a deer and pronounced "gä." So with a combination of a horse and a deer we have "bä-gä" as close to "bar-ger" as the Japanese could get. However, when "bä-gä" is slurred together without the dash, it is a scathing and insulting epithet, literally meaning "darn fool." You probably read in the newspapers that it was reported that the Premier of Japan referred to a member of the Japanese Diet by using "baga" and as a result a no-confidence vote occurred and new elections were held in Japan.

Fig. 3.—A rice-field worker showing his rain hat and coat and implements.

Anyhow you now have some idea of my experience with the Japanese people. It is very limited. I want you and them to judge everything I say on the basis of that limitation.

One of the first questions that everyone asks is, "How did you like the Japanese?" I believe that this query can best be answered by pictures. The charming group of small flower-bearers (Fig. 2) met the mission on the first stop. There was one flower-bearer for each member of the mission. Here in this picture you sense the almost unbelievable cordiality and hospitality of the Japanese people. This statement is not singularly from the mouth of an overwhelmed newcomer; it was on the lips of every American of the civilian and military occupational forces with whom I talked.

Western dress appeared to be used by about three out of four Japanese in the cities. Even the poorer Japanese often appear in Western garb.

On the road to Nara, the most ancient capital of Japan, we paused to take a picture of a rice field and the peasant who was tending it (Fig. 3). He wears his raincoat on his back because he is stooping over almost all the time he is working in the fields. He may have owned the field, as the American Occupational Forces put through a drastic land reform program by which the government of Japan bought the land from its owners and resold it in small plots to the peasants on very liberal terms.

There are now seven dental schools in Japan, four in Tokyo, two in Osaka, and one in Kokura. One of the recommendations that the Mission[3] made was that every college of dentistry should be made an integral part of a recognized university as soon as possible. Also, that the dental school or dental college in the university organization should have the same academic status as that of other colleges in the university because dental education is relatively as important to human welfare as any other phase of education.

Fig. 4.—Unrestored Municipal Industrial Exhibit Building which has been left as a monument to blast-devastated Hiroshima.

One of the most striking and perhaps typical dental restoration that one saw everywhere was the chromium-nickel or stainless steel crown. A simple interproximal cavity is often treated by removing a little enamel and then one of these hideous crowns is placed on the tooth. It was a bit disconcerting to have a fine-looking person flash an all-chromium smile just as it was in this country forty or fifty years ago when one might see an all-gold smile. Devitalization with arsenic is also said to be widely practiced, and judging by the fearful discoloration of so many front teeth, not much of the pulp was removed or it was removed much later than it should have been.

A question that one is often asked is, "Did you see Hiroshima or Nagasaki, the two cities destroyed by atomic bombs?" We visited Hiroshima, and much to our amazement it has been almost completely restored. It is almost inconceivable how these industrious people could recreate Hiroshima in such

a short space of time. No wonder we were repeatedly told by the American Occupational Forces that they felt like helping the Japanese in every possible way because the Japanese were willing to help themselves.

In Hiroshima there is a medical research center, jointly sponsored by the American and Japanese governments. Surprisingly enough there have been few if any dire results of the atomic radiation in the new generation. Some had anticipated human anomalies, that is, human beings with a marked deviation from the normal standard. This has not been demonstrated to date.[4] Demonstrations of late body effects have been shown in a few of the relatively heavily irradiated survivors; cataracts[5] and leukemia,[6] for example.

Fig. 5.—The Torii Gate to the Shinto Temple at the resort island of Miyajima.

Fig. 6.—Kazuo Sato (D.D.S., Chicago College of Dental Surgery, Loyola University, 1901, and M.D., Rush Medical College, 1903, now University of Chicago), president of the Japan Dental Association at the time of the Mission's visit.

One of the few biologic differences that can be noted is the shortening of the roots of the teeth in children who were irradiated during the tooth formative period. This shortening is pronounced, amounting to as much as 50 per cent of the normal root length. Much the same phenomenon is observed in American children who have had roentgen therapy for neoplasms about the head and neck during the formative period of the roots of the teeth.

The structure in Fig. 4 was the Municipal Industrial Exhibit Building. It has been dedicated as a monument to this blast-devastated city. The bomb exploded almost directly over the spot at a height of about one-half mile. Notice the terrible destruction which this building took even though it was not leveled. One wonders how the tree on the left survived. Next door, to the right, is a Buddhist cemetery. Here the priest showed us how the surface of the marble tombstones was vaporized by the heat of the blast except where a shadow would be cast by an upright part of the tombstone. There the original polish of the stone was not disturbed. Some individuals survived the blast even though they were near the center because they were protected by an opaque object that cast a dense shadow or were in basements or sub-basements.

Now we will go from the ugly to the beautiful. The photograph of the Torii (Fig. 5) gate to the Shinto temple at the island resort of Miyajima was taken in the rain late in the afternoon. It is one of those scenes that make people exclaim that Japan is a picture-book country populated by picture-book people. Speaking of people, one of the Mission's gracious hosts was the president of the Japan Dental Association, Kazuo Sato (Fig. 6) (D.D.S., Chicago College of Dental Surgery, Loyola University, 1901, and M.D., Rush Medical College, 1903). He is, as his picture indicates, a wise, charming, and revered gentleman.

Fig. 7.—Original of the three world-famous monkeys which are on the walls of one of the buildings in the Tosho Temple Grounds at Nikko.

The Mission visited the Imperial Summer Palace Grounds at the shrine city of Nikko. Prior to the Occupation these were not open to the Japanese public. Without fail the Japanese always mentioned this when we had access to formerly forbidden properties. Such scenes as this were for Imperial eyes only—but the Japanese eyes have been opened—we hope far enough to make democracy work.

The shrine city of Nikko is a picture-book place in the fullest sense of that phrase. The buildings on the Tosho Temple Grounds are beautifully colored, more than the Japanese like. They feel that the Chinese Buddhist influence was too great here. The gate house to the Tosho Temple Grounds provides as beautiful a display of the ornate architecture of the Orient as there is.

Perhaps it would be well to close this rambling account with the picture (Fig. 7) of the original of these three world-famous monkeys, which adorn one wall of a building in the Tosho Temple Grounds at Nikko. The philosophy of these three monkeys—hear no evil, speak no evil, see no evil—is as appropriate today as it was on that day centuries ago when the Japanese artist created them.

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III. Abstracts of Papers Read in General Meeting Sessions

1. Oral Lesions in Experimental Vitamin B Deficiencies in Adult Dogs. *D. Afonsky, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Niacin, riboflavin, pyridoxine, pantothenic acid, and folic acid deficiencies were induced in adult mongrel dogs by means of the synthetic diet (*J. D. Res.* **32**: 633, 1953). The period of observation lasted from 200 to over 400 days. A nonspecific hyperemic gingivitis associated with calculus accumulation was present in every animal. It could be prevented by brushing the teeth with a toothbrush and water. In none of the dogs was the condition pronounced enough to justify the diagnosis of periodontal disease. Acute ulcerative stomatitis was observed in niacin and pyridoxine deficiencies. Fusiform bacteria and spirochetes were the predominant microorganisms. The most characteristic oral lesions in all the deficiencies were those of the

tongue. Degenerative changes in the lingual papillae were found in all animals. Niacin, pyridoxine, and riboflavin deficiencies, in particular, were accompanied by extensive atrophy of the lingual papillae.

2. Determination of Fluoride by Diffusion of Hydrogen Fluoride. *W. D. Armstrong and Leon Singer, Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.* Separation of fluoride from other ions in the sample is effected by diffusion of hydrogen fluoride from the acidified sample in polyethylene bottles. The evolved hydrogen fluoride is collected by reaction with sodium hydroxide on a strip of polyethylene contained in the diffusion bottle or, alternatively, in a solution of sodium hydroxide. The method has been applied with good results to the micro determination of fluoride in bone, in fluoride-free bone, and in aqueous solutions in the presence of urea, glucose, sodium carbonate, and sodium chloride. The new procedure has the advantage of simplicity and ease of operation over distillation of fluosilicic acid, the procedure now generally used for separation of fluoride from interfering substances.

3. Alkaline and Acid Phosphatases in the Developing Teeth. *James K. Avery, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* An investigation concerned with the presence of the enzymes, alkaline and acid phosphatases, was carried out on the fetuses of the human being and the domestic pig. The jaws were serially sectioned in the sagittal plane and the developing teeth were studied from the time of their initiation until the time of eruption. Decalcification of the calcified structures was carried out with formic acid-sodium citrate (Greep, Fischer, and Morse, 1947), and versene. A series of tissues subjected to decalcification were compared to a control series prepared in the same manner without decalcification to estimate the enzyme activity lost. No apparent loss of enzyme activity was noted when the decalcification process did not exceed seven days. By these means it was thus possible to compare quantitatively the activity of these substances within the dental tissues over the entire period of tooth development. Alkaline phosphatase appears in greatest concentration in the stratum intermedium adjacent stellate reticulum and the dental papilla near the odontoblasts in early tooth formation. After dentinogenesis has begun it appears more concentrated in the odontoblast. The ameloblast appears essentially negative until the time of final calcification of the enamel matrix. Acid phosphatase distribution is similar except that it appears in concentration in the ameloblast during the entire period of amelogenesis.

4. The Amino Acid Content of Human Enamel. *Gino Battistone and George W. Burnett, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* The amino acid content of human enamel has been determined by qualitative and quantitative two-dimensional paper chromatography. Enamel for analysis was obtained from permanent caries-free teeth by two methods: (1) by grinding whole teeth to +60, –100 mesh and separating the enamel from dentin and cementum by floatation methods; (2a) by chipping enamel from dentin and grinding any dentin from the enamel chunks; (2b) by grinding the chipped enamel obtained by method 2a to +60, –150 mesh. These samples of enamel were decalcified in 15 per cent disodium versenate (pH 7.5), dialyzed against water, and dried to constant weight at 100° C. Portions of the decalcified enamel were hydrolyzed in sealed bombs with 20 per cent HCl at 125° C., while others were hydrolyzed in 5N NaOH at 115° C. for varying periods of time. After hydrolysis all samples were oxidized with H_2O_2. Enamel obtained by method 2a had a lower ash and more reproducible amino acid content than that obtained by the other methods. The percentage composition of the amino acids of

enamel obtained by method 2a was found to be as follows: histidine, 0.6; lysine, 2.5; arginine, 7.0; aspartic acid, 5.2; alanine, 9.6; cystine, 0.6; glutamic acid, 10.1; glycine, 19.65; hydroxyproline, 5.1; methionine, 1.0; proline, 4.0; serine, 6.4; threonine, 4.4; tryptophane, 0.1; tyrosine, 1.0; valine, 3.4; phenylalanine, leucine, and isoleucine (determined quantitatively as a group), 13.0. The ash content of versene decalcified enamel was 5.86 per cent.

5. Gingivitis Not Reduced by Chlorophyll Compounds in Dentifrices. *Wilfred B. Bell, Samuel O. Huddleston, Dan Y. Burrill, and William R. Wolfe, University of Louisville, School of Dentistry, Louisville, Ky.* The anterior teeth and gingiva of 109 subjects, aged 12 to 30, with fair to good oral hygiene, were photographed in color. Each subject was supplied with a standard paste dentifrice to which had been added either copper chlorophyllin (forty-eight subjects), potassium iron chlorophyllin (seventeen subjects), or a food dye producing the same green color (forty-four subjects). Throughout the experiment each subject continued his regular oral hygiene procedures, using only the dentifrice supplied. Each mouth was rephotographed at the end of one month and again at the end of two months. For evaluation the projected image of the initial photograph of each patient was kept on the screen and the image of each later photograph was projected beneath it so that direct comparison could be made. From the projected photographs three judges simultaneously but independently evaluated gingival conditions. Initially there was gingivitis in the anterior of every mouth. The later photographs seemed to show no advantage for any dentifrice. The changes in the amount of gingivitis were approximately the same for all dentifrices. Changes were slight if present and apparently random. The chlorophyll compounds used appeared to add nothing of value to the dentifrice for the control of gingivitis as it occurred in the mouths of these subjects.

6. Histological Evaluation of Pulpotomy. *Harold Berk and M. Michael Cohen, Department of Oral Pediatrics, Tufts College Dental School, Boston, Mass.* Vital pulpotomies were performed on seven noninfected primary and permanent teeth that were to be extracted for orthodontic reasons. These were in patients 9 to 12 years of age, and the teeth were observed after five to six weeks in four patients and after fifty-two weeks in the fifth patient. The six experimental pulpotomies that were treated with calcium hydroxide and calcium hydroxide methyl cellulose paste showed bridges of dentin below the pulpotomies. These bridges were tubular and of considerable thickness and sealed off the remaining pulp stumps. Trapped fibroblasts were observed in the dentin bridges. Below the dentin bridge, a single layer of odontoblast-like cells were found. The remaining portion of the pulp stump appeared normal and showed an increase in fibroblasts. The one control tooth was treated with zinc oxide and eugenol. It showed extensive necrosis of the remaining pulp tissue and hydropic degeneration of the odontoblastic layer in contrast to the experimental tooth in the same mouth.

7. Artificial Caries. *F. C. Besic, Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* A definition is presented of the chemical and bacteriologic conditions under which enamel changes produced in vitro closely resembling those of clinical caries. The two types of lesions have been compared in respect to several histologic and chemical properties and appear to be substantially identical.

8. The Physical and Histological Aspects of the Brown Spots. *B. R. Bhussry and V. M. Emmel, Division of Dental Research, School of Medicine and Dentistry, Rochester, N. Y.* The physical, histological, and chemical aspects of brown spots in enamel which occur on the proximal surfaces of the teeth have

been studied. The areas chosen were clearly demarcated by brown color, not affected by scaling, and presented no break in the enamel surface. Extracted teeth of known age and clinical history were used for the study. Sagittal sections 50 to 75 microns thick were prepared through predetermined areas. Histological examination of these sections suggests the presence in these areas of a brown material in addition to the normal organic components of enamel. The bulk of these pigmented areas do not fluoresce in contrast to the brown pigmentation found elsewhere in enamel. Controlled decalcification of these sections under the microscope, using 1 per cent formic acid in 10 per cent formalin at pH 5 showed that they were resistant to decalcification. Preliminary microhardness determinations by means of a Tukon microhardness tester indicates that the hardness index in the brown area is only about one-third of that in normal enamel. Kjeldhal determinations give a higher nitrogen content in the brown spots than in the normal enamel of the same tooth, indicating a higher percentage of protein. The origin and nature of the organic material in these areas are still unknown. It is hoped that an extension of the present studies will provide an insight into these important questions.

9. Studies Concerned With the Effects of Fluorides on the Blood Vascular System. *Jack G. Bishop, Alfred W. Richardson, and Joseph C. Muhler, Indiana University, Bloomington, Ind.* Whereas vasomotor disturbances have been reported to result from fluoride intake of toxic proportions, little has been done to assay the effect of fluorine on the vascular system when administered in subtoxic quantities. It has been found in our studies that blood fluorine concentrations of less than 1 mg. per liter of blood have a significant dilator effect on the vascular bed, there being considerable variation with different fluoride salts studied. The rate of volume blood flow was measured by a sensitive linear electromagnetic bloodflow meter, and blood pressure transients by a strain gauge, amplifier, and ink-writing recorder. Peripheral resistance was calculated from these measurements by the PRU method of Green. Six to nine dogs, 8 to 17 kilograms, were used for each variable study, pentobarbital sodium and heparin being used in the operative procedures. Peripheral dilatation and increased blood flow could be readily demonstrated following the intravascular injection of 0.15 mg. or more fluorine, the action being more prolonged than typical with most vasodilator agents. The action was progressive up to about 1.0 mg. without observable compensation, but greater administered quantities produced partial but ineffective compensation with some tachyphylaxis. Some fluorides were more effective dilators than others, the relative order of effectiveness being SnF_2, KF, Na_2PO_3F, and NaF among the compounds studied. It may be possible later to show that at least a part of the action of administered fluorine may be to induce increased salivary flow, via vasodilatation, for its therapeutic dental properties.

10. Lack of Effect of Fluoride Ingestion on Mammary Carcinoma Development in Mice. *J. J. Bittner, W. D. Armstrong, and Leon Singer, Division of Cancer Biology and Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.* Female ZD_2F_1 hybrid mice were kept as virgins and given drinking water containing 0, 5, or 10 ppm of fluoride beginning with the twenty-eighth to fortieth day of life. After sixteen months of the experiment no difference in mammary carcinoma incidence or age of development of tumors has appeared among the animals in the separate groups.

11. The Effect of Castration and Desalivation on the Endocrine Glands, Body Weight, Metabolism, and Dental Caries in the Rat. *David Bixler, Joseph C. Muhler, and William G. Shafer, Department of Chemistry and*

School of Dentistry, Indiana University, Bloomington, Ind. In an effort to explain some of the effects of desalivation and castration upon the endocrine glands and normal body metabolism, 300 animals were divided into eight different experimental groups and placed on a cariogenic diet. The first group was desalivated, the second had a sham desalivation, the third was castrated at 30 days of age, the fourth and fifth were castrated at 60 days (one receiving a noncariogenic diet until operation), the sixth and seventh were both castrated and desalivated and, in addition, seven received natural sex hormone supplements, and the eighth was unoperated and served as controls. Daily food and water consumption was measured in groups one and two and the controls. After 140 days the animals were sacrificed, the heads examined for caries, the femurs analyzed for fluorine, calcium, and phosphorus, and the endocrine glands removed, weighed, and fixed for histologic study. Desalivation had no significant effect on adrenal weight, the testis weights of operated animals were considerably smaller, and uterine weights noticeably increased. Castration resulted in an increased adrenal weight in males, decreased weight in females. Hormone-injected animals had organ weights similar to controls. The 60-day castrates did not show as extensive uterine atrophy as the 30-day castrates. Desalivated animals ate less food and drank less water than either the sham-operated animals or controls. Oxygen consumption studies indicated that general body metabolism of desalivated animals and animals receiving fluoride supplements was much lower than that of control or castrated animals. Castration appeared to reduce caries in males. Castrated and desalivated animals had much higher caries scores, while those receiving hormones had the highest score. Castration at 30 days was more effective in reducing decay than at 60 days, and the males on the noncariogenic diet for sixty days had the lowest caries scores. Sham-operated and control animals had about equal caries scores, while desalivates had pronounced dental caries.

12. Amino Acids in Dental Plaque. *R. Q. Blackwell, L. S. Fosdick, and I. Namajuska, Chemistry Department, Northwestern University Dental School, Chicago, Ill.* Qualitative paper chromatography was used to study the free amino acids present in individual plaque samples from caries-active and caries-inactive individuals. Pooled plaque samples from normal and from highly caries-active individuals were studied quantitatively for free and total amino acid content by ion exchange column chromatography. All samples were extracted with 75 volume per cent ethanol to remove free amino acids. In the pooled plaque the residue was hydrolyzed with 6 N hydrochloric acid and the amino acid content of the hydrolysate determined. Individual plaque samples showed some variation in detectable quantities of amino acid on paper but no consistent difference was noted between caries active and inactive. Most frequently found were arginine, alanine, aspartic acid, glutamic acid, glycine, and valine. Leucines and proline were usually present. A spot believed to be delta-amino valeric acid was found in all caries active and some inactive plaques. Occasionally present were lysine, serine, taurine, tyrosine, and three unidentified spots. (Histidine, tryptophan, threonine, and phenylalanine were found in small quantities in pooled samples and presumably were also in the individual samples but in insufficient quantities to appear on the chromatograms.) Quantitatively the pooled plaque samples contained 0.25 per cent free amino acid in the normal and 0.19 per cent in the caries active. The total amino acid content of the normal plaque was 5.76 per cent, which is roughly one-half the total organic content of the plaque. Present in greatest amounts were glutamic acid, aspartic acid, proline, arginine, alanine, and lysine. It was suggested that the buffering action of the plaque in part may be due to its protein and amino acid content,

particularly to the distal carboxy groups of aspartic and glutamic acids (both free and in protein) which have pK values of approximately 4 and would offer increasingly effective buffering as the plaque pH drops to 5 and below.

13. Effect of Fluorides Upon the Permeability of Intact Dental Enamel to Sodium Ions. *Harold E. Brewer, Joseph C. Muhler, and Robert B. Fisher, Department of Chemistry and School of Dentistry, Indiana University, Bloomington, Ind.* Since it has been shown that all fluorides are not similar in their ability to decrease powdered enamel solubility or reduce the incidence of dental caries in experimental animals, it is deemed advantageous to study the role of fluorides in the permeability of the intact enamel shell. Intact enamel shells are prepared by sectioning human teeth at the cemento-enamel junction, removing coronal dentin, sealing the open cervical region with impermeable wax, and inserting a small bore capillary tube into the sealed enamel shell. A microscopic, crystallographic test for detection of sodium ions was adapted for special use in this experiment: drops of test solution were evaporated to dryness on a microscope slide and to the resulting residue was added uranyl acetate to form characteristic crystals with sodium ions. The optimal time for passage of sodium ions through the intact dental enamel shell, and the effect of treatment of the outer enamel layers with various fluorides, indicate that the enamel shell is permeable and that the rate of permeability is dependent upon these factors and, in addition, on the osmotic pressure of solutions inside the enamel shell.

14. Distribution in Depth of Fluoride in Human Enamel. *Finn Brudevold, Dwight E. Gardner, and Frank A. Smith, Eastman Dental Dispensary and University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* Successive layers of surface enamel, from erupted teeth of persons from the following age groups: under 20, 20 to 29, 30 to 49, and over 50, were ground and pooled. Similar enamel samples were obtained from unerupted teeth and from mottled teeth of a group of persons of unknown age. Chemical analysis showed that the external enamel of the erupted teeth had a concentration of fluoride six to ten times greater than that of the bulk of the enamel. The fluoride content of the surface enamel was smallest in the under 20 group (600 ppm) and reached a plateau of 800 to 1,000 ppm in the older age groups. The increase in the fluoride content was confined to the external enamel, demonstrating that there is only a slight penetration of fluoride from the surface. A high concentration of fluoride was also found in the external enamel of impacted teeth. Therefore, appreciable amounts of fluoride may be picked up from tissue fluids by the superficial enamel prior to eruption. The pattern of distribution of fluoride in mottled enamel was the same as that of the other teeth, but the concentration of fluoride in the different layers was about twice as great.

15. A Study of the Effect of the Addition of Certain Amino Acids to Enamel-Decalcifying Buffers. *Michael G. Buonacore and Biruta Sommer, Eastman Dental Dispensary, Rochester, N. Y.* Simple buffers of acetic, lactic, citric, and pyruvic acids at pH 4 were prepared. The dissolving effect of 20 c.c. of these solutions on 100 mg. of powdered enamel was tested. The remainder was then divided into four portions and to each part was added enough aspartic, glutamic, valine, or alanine to give a 0.1N concentration. These solutions were then readjusted to pH 4 as required and 20 c.c. portions tested for their effect on powdered enamel. Results show that the addition of glutamic, alanine, and valine to each buffer produced little change in its ability to dissolve enamel. The addition of aspartic acid, however, significantly reduced the ability of each buffer to dissolve enamel. In terms of milligrams of enamel dissolved, the results are: buffer alone—acetic, 36.5;

lactic, 29.6; citric, 29.1; pyruvic, 24.6; buffer plus aspartic acid—acetic, 19.4; lactic, 20.5; citric, 21.4; pyruvic, 20.5. The existence of aspartic acid as one of the free major acid components of carious dentin has been reported (Atkinson, H. F., and Matthews, E., *Brit. D. J.* **86**: 142, 1949). It is suggested that the presence of aspartic acid or its salts in the acidic buffer system of plaques or carious material may render them less destructive toward tooth substance.

16. Spectrochemical Analysis of Enamel and Dentin From Syrian Hamsters. *George W. Burnett and Ralph R. Lobene, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* A quantitative spectrochemical analysis was made of separated, ashed enamel and dentin from incisor and molar teeth of adult male and female Syrian hamsters. Special methods, developed by the National Bureau of Standards, made possible quantitative determinations of the elements in the range of 0.0001 to 0.5 per cent in the 2,200 to 4,800 Å range of the spectrum. After the limit of detection was established for each element, observations were made for thirty-three elements, of which seventeen were found in the tissues. The percentage composition of the silver, aluminum, boron, barium, calcium, chromium, copper, iron, magnesium, manganese, sodium, nickel, phosphorus, lead, silicon, tin, and strontium of the ashed tissues was then determined. Calcium and phosphorus were the major metallic constituents of the tissues, with only the minor constituents, magnesium, sodium, and iron being present in more than 0.1 per cent. Magnesium concentration was lowest in enamel and highest in dentin; sodium concentration was more consistent in the various tissues; iron concentration was highest in incisor enamel from both males and females, less in dentin from incisors of males, similar in molar enamel and dentin from both males and females, and least in molar dentin from females. Composition differences between the tissues were also noted for chromium, manganese, nickel, and silver. The concentration of other elements that were detected showed no significant differences between the various samples of tissue.

17. Histochemical Demonstration of Mucin in Normal and Neoplastic Salivary Glands. *M. S. Burstone, Department of Anatomy, University of Chicago, Chicago, Ill.* Submaxillary and sublingual glands of ten adult rats and twelve human salivary gland tumors were used in this study. Some of the tissues were fixed by freezing and drying while others were fixed in chilled acetone-ethanol. The tissues were infiltrated and embedded in paraffin and sectioned at 6 microns. Mucins were visualized by the following method which has not been previously used to the author's knowledge: Sections were nitrobenzoylated for four hours with 5 per cent p-nitrobenzoyl chloride in dry pyridine. Sections were reduced, diazotized, and then coupled with 1-amino-8-naphthol-3:6-disulphonic acid. Cytoplasm of cells of the sublingual gland of rats stains an intense red violet. Serous gland cytoplasm does not stain as intensely and exhibits shades toward the violet. So-called "special serous" cells of the rat exhibit a very pale-staining cytoplasm. Preacetylation (with acetic anhydride) of sections abolishes the staining of sublingual mucin but not of submaxillary mucin. Prebenzoylation (with benzoyl chloride) of sections blocks the reaction completely, thus suggesting that nitro-benzoylation under specified conditions will essentially involve amino acids. Mucins of salivary gland tumors stain, and exhibit varying states of reactivity. The use of histochemical procedures in conjunction with pretreatment with certain "blocking" agents may serve to characterize the different types of salivary mucins.

18. Control of Post-Mortem Changes in Radioiodine Distribution in Teeth. *Byron G. Butt and J. D. Hauptfuehrer, College of Dentistry, Univer-*

sity of Illinois, Chicago, Ill. The possibility of changes in tracer distribution following the sacrifice of experimental animals was studied in rhesus monkeys. The first animal was injected I.P. with carrier-free NaI^{131} and the teeth removed after two hours. Several teeth were treated in each of the following ways: freezing-dehydration, freezing in liquid nitrogen without dehydration, storage at –5° C., and storage at room temperature. The teeth were sectioned and radioautographed to visualize the pattern of radioactive iodine distribution. Radioautographs of sections of those teeth which were stored at room temperature showed diffuse penetration of dentin. The three frozen groups showed the highest uptake of tracer to be in the circumpulpal dentin with a gradual decrease in the centrifugal direction. Near the dentoenamel junction, in the area of the dichotomy of dentinal tubuli, more radioiodine was apparent. Another monkey was treated in the same manner except that the time between isotope administration and sacrifice increased to twenty-one hours. In contrast to the specimens obtained two hours after iodine administration, all teeth from this animal whether frozen or stored at room temperature showed diffuse penetration of dentin. The distribution of iodine in these teeth appeared the same as in the teeth stored at room temperature in the short-term experiment. It is concluded that redistribution of radioiodine takes place in short-term experiments if freezing storage is not used.

19. The Site of Action of Sodium N-Lauroyl Sarcosinate on the Glycolytic Scheme. *J. A. Carbon, R. Q. Blackwell, and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* When incubated with fresh saliva, sugar, and finely powdered dental enamel, sodium N-lauroyl sarcosinate has recently been shown to reduce acid formation and calcium dissolution significantly. It was postulated that this inhibition is due to blocking of one or more of the enzymatic reactions in the Embden-Meyerhof pathway, thus preventing oral bacteria from degrading sugars to acidic products. At a concentration of 0.00085 M, this compound inhibits sugar degradation by active yeast cells approximately 50 per cent, while a 0.0017 M concentration gives over 90 per cent inhibition. Studies on phosphate uptake and glucose disappearance as a function of time for intact yeast cells show that the preliminary phosphorylation reactions are strongly inhibited, particularly the hexokinase reaction. Indications are present that other phases of the glycolytic scheme may be similarly inhibited.

20. The Effect of Topical Fluoride on the Dental Caries Experience in Adult Females of a Military Population. *Wm. J. Carter, Philip Jay, Irving Shklair, and Lewis Daniel, Research Departments, Dental Department, Great Lakes, Ill., and the School of Dentistry, University of Michigan, Ann Arbor, Mich.* Sixty Naval enlisted women were treated topically with sodium fluoride, and eighty-eight were treated with sodium chloride as a control group. Four tables of statistics are presented to show the results of this form of treatment on the dental caries experience one year following treatment. There was no significant reduction in caries incidence of persons receiving a neutral 2 per cent sodium fluoride treatment over those receiving a placebo. The percentage reduction of caries exhibited in teeth and tooth surfaces was not statistically significant. The salivary lactobacillus counts were not affected by the fluoride ion.

21. Proteolytic Enzymes of Human Saliva. *H. H. Chauncey, V. M. Johnson, and V. F. Lisanti, Tufts College Dental School, Boston, Mass.* In the determination of the proteolytic enzymes of whole saliva, activity was demonstrated at both acid and alkaline pH. An active principle was found to be associated with the salivary debris and is probably due to the bacteria and other cellular components normally present. The enzymatic liberation of

acid-soluble products measured as tyrosine was used to determine the rate of degradation of casein substrate at pH 7.4 and hemoglobin substrate at pH 4.0 and 7.5. In addition, an inactive proteolytic enzyme was found in the supernatant fluid of centrifuged whole saliva, which appears to be similar to the serum protease, plasminogen, activation occurring on the addition of a kinase. The salivary protease is capable of hydrolyzing both hemoglobin and fibrin. Activity was measured turbidimetrically using a newly developed fibrin substrate. The presence of protease activity in parotid saliva has been demonstrated. The best results were observed on the euglobulin fraction obtained by dialysis and ammonium sulfate precipitation of the parotid secretion. The optimum pH and substrate concentration as well as the effect of known activators and inhibitors of the whole saliva protease was determined. A comparison was made of the enzyme characteristics of serum plasmin and the activated salivary protease.

22. Transplantation of Developing Teeth. *Henry B. Clark, Jr., Joseph C. Tam, and David F. Mitchell, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Nineteen incompletely formed lower third molar teeth were transplanted to first molar spaces in the same jaw in eighteen human subjects ranging in age from 13 to 23 years, with an average of 16.5 years. During periodic observation all became firm, comfortable, and retained good color. Positive electric vitality tests resulted in 43 per cent of fourteen cases tested by the seventh postoperative month and 84 per cent of thirteen cases tested at the tenth month. Radiographs indicated a well-formed periodontal membrane and lamina dura and no evidence of periapical or periodontal pathology but did disclose a reduction in size of the pulp chamber. The extent of eruption achieved was disappointing. The maximum root growth, estimated from radiographs, was 2.5 mm. and the average 0.9 mm. Proper position in the arch was not reached in any instance when the transplant was buried deeply (thirteen cases). Greatly improved position resulted when they were placed about 1.0 mm. below the occlusal plane of the adjacent teeth and immobilized with a splint (six cases). Two of the former group were so malposed they were extracted and studied microscopically. Both showed vital pulps, with no evidence of inflammation. In addition to the normal primary dentin, presumably formed before transplantation, a very thick layer of irregular dentin had been deposited and predentin was present. Cementum and periodontal membrane fibers were seen. In this study transplantation of incompletely formed teeth was followed by reattachment of periodontal structures and retained vitality of pulp, but pulp chambers were reduced in size by a heavy deposit of irregular dentin.

23. A Comparison of Procaine, Lidocaine, and Unacaine. *John P. H. Clark, School of Dentistry, Western Reserve University, Cleveland, Ohio.* Potencies of epinephrine-free 1 per cent solutions of procaine, lidocaine, and Unacaine were compared by utilizing abdominal field blocks on guinea pigs. Thirty-six injections were given with each agent. The test sites received electric stimuli immediately after injection and at twelve-minute intervals. At the first postinjection stimulation, the pain threshold had been increased by procaine 41 per cent, Unacaine 44 per cent, and lidocaine 55 per cent. The times of maximum increase in pain threshold were: procaine 49 per cent at twelve minutes, Unacaine 58 per cent at twelve minutes, and lidocaine 70 per cent at thirty-six minutes. The increase in pain threshold fell below 5 per cent in sixty minutes for procaine, in eighty-four minutes for Unacaine, and in one hundred eight minutes for lidocaine. Two clinical studies were conducted; 100 pairs of injections were given in each. Two per cent procaine with epinephrine 1/20,000 and 2 per cent lidocaine with epinephrine

1/50,000 were compared. Anesthesia occurred in 3.5 minutes with procaine and 2.1 minutes with lidocaine. The duration was eighty-four minutes for procaine and 118 minutes for lidocaine. Two per cent lidocaine and 2 per cent Unacaine each with epinephrine 1/50,000 were compared. Anesthesia occurred in 2.3 minutes with lidocaine and 3.0 minutes with Unacaine. The duration was 106 minutes for lidocaine and fifty-one minutes for Unacaine. Intraperitoneal injections on white mice indicated that the approximate M.L.D. 50's expressed in milligrams per kilogram of body weight were: lidocaine 138, procaine 215, and Unacaine 595. The data indicate that lidocaine is most potent, with Unacaine intermediate and procaine least potent. Procaine and Unacaine are similar in duration of action; lidocaine is much longer acting.

24. A Rapid Quantitative Laboratory Test for Salivary Amylase. *C. K. Claycomb, Marcia M. McChesney, and Marshall L. Snyder, University of Oregon Dental School, Portland, Ore.* Previously developed quantitative tests for amylase were unsuitable for the analysis of large numbers of saliva specimens. A simple, rapid photometric method was developed with which it is possible to make approximately twenty-five determinations per hour. This test is based on the intensity of the blue color formed by iodine in the presence of starch remaining after incubation with amylase, and compared with a control without enzyme. Alpha amylase was used to determine optimum starch, iodine, incubation time, and temperature to give a linear response. With the concentrations selected, reproducible results were obtained with serial dilutions of either commercial amylase or saliva. For our purposes, 0.5 ml. of saliva was incubated at 40° C. with 250 mg. of starch in a phosphate buffer of pH 7.0, and at the end of five minutes enzymatic activity was arrested and color developed by the addition of HCl-I mixture. The percentages of light transmission obtained were taken as an index of amylase activity based upon prior standardization studies. In 421 tests, 360 or 86 per cent fell within 0 to 40 per cent transmission, and of these, 271 or 64 per cent were within 10 to 20 per cent transmission. The inherent error of the instrument (4.0 per cent) at the low range was recognized, but it seemed to have no significance. There was no correlation between the increasing amounts of salivary amylase activity and increasing numbers of lactobacilli or positive Snyder tests in the respective specimens of saliva.

25. Oral Conditions of Primitive Bushmen of the Northern Kalahari Desert. *A. J. Clement, J. Plotkin, and L. S. Fosdick, University of the Witwatersrand and South African Institute for Medicail Research, Johannesburg, Union of South Africa, and Northwestern University Dental School, Chicago, Ill.* The incidence of dental caries, periodontal disease, abrasion, and oral hygiene conditions of the Bushmen were made by clinical examination. In addition to the dental aspects, laboratory tests on saliva were performed. Snyder's tests and lactobacillus counts were made on the saliva. The pH of the dental plaques and the concentration of lactate in the plaques were determined before and after a thirty-second sugar rinse. It was found that the Bushmen were practically caries free. The Snyder's test and lactobacillus counts were negative. Abrasion and gingival disturbances were marked. The pH remained in 83 per cent of the cases for twenty minutes after a sugar rinse. The neutralized and free lactic acid were determined by means of the Henderson-Hasselbach equation. In no case was enough free lactic acid present to cause decalcification of the teeth.

26. Brookline, Massachusetts Pre-School Dental Survey. *M. Michael Cohen and Leonard B. Green, Brookline Health Department, Brookline, Mass.* The survey included a DEF (decayed, extracted, filled) evaluation on 4- and

5-year-old children. In addition the incidence of gingivitis and gingival enlargement was observed and the occurrence of malocclusion was recorded. In 347 4-year-old children 68 per cent had one or more DEF teeth. The average number of DEF teeth per child was 2.89 while the average number of filled teeth per child was 0.86. Of eighty-eight 5-year-old children 78 per cent had one or more DEF teeth. The average number of DEF teeth per child was 4.06 while the average number of filled teeth per child was 0.81. Hypoplastic defects were observed in thirteen children. One hundred thirty children showed marginal gingivitis. Often the gingivitis was accompanied by gingival enlargement. Stippling was seen in practically all children with normal gingiva. In 443 children, 393 showed a Class I occlusion with a wide variation of the anterior overbite, from an edge-to-edge bite relationship to a deep overbite, while the posterior occlusion was consistent with the "occlusial relationships" described by Angle. In the Class I group, 103 children had oral habits and the anterior teeth showed an anterior open bite which varied from a slight opening to 5 mm. or more. Forty-seven children showed a Class II malocclusion and twenty-seven in this group had oral habits. Only three children showed a Class III relationship and one in this group had an anterior crossbite. Of the 443 children examined 29.5 per cent had indulged in oral habits.

27. Application of Freeze-Dried Bone Grafts in Cysts of the Jaws. *Donald E. Cooksey, School of Dentistry, Georgetown University, Washington, D. C.* A series of clinical and animal experiments were carried out to determine whether or not freeze-dried bone would be a suitable material for employment in grafts to repair defects caused by cysts of the jaws. Interest in the material resulted from the following advantages: (1) it can be stored for indefinite lengths of time without special precautions as to refrigeration, sterility, or effects of light, if retained in the original container; (2) no second operation is required as would be necessary if fresh autogenous bone were employed; (3) the operator need not depend upon a near-by banking facility for supply. The following results have been obtained with freeze-dried bone: (1) it is well tolerated in both cortical and cancellous form in human jaws; (2) it is eventually absorbed and replaced by normal bone; (3) incidental oral contamination does not appear to affect the material in the mouth; (4) immediate prosthesis may be worn successfully over the graft; (5) it has no tendency to slough or sequestrate once established; (6) it was retained in all cases when placed in experimental animals (dogs) and there was gross and histologic evidence of healing.

28. Chemical Aspects of Caries of the Enamel. *T. B. Coolidge and M. H. Jacobs, Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* In order to clarify the chemical processes involved in clinical caries a comparison has been made of the chemical, bacteriologic, and histologic changes occurring in artificial and clinical caries.

29. A Non-Carious Cavity in the Second Upper Molar of Rats. *Gerald J. Cox and Sara F. Dixon Goodlin, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* In the system of recording cavities in rat teeth (Cox and associates, *J. D. Res.* **18**: 469, 1939), provision was made for recording "atypical" lesions. In general these lesions were regarded as fractures, except for holes which appeared in the distal cusp of the upper second molars. These latter were regarded as true carious lesions. However, in later studies rats weaned at 21 days and placed on a high-fat diet for three weeks showed these lesions but no others in any areas. The composition of the high-fat diet was: lactalbumin 30, sucrose 12, Crisco 26, oleomargarine 26, brewer's yeast 5, Osborne and Mendel salt mixture 6, and haliver oil 10 drops per kilogram.

There were no characteristics of carious attack observed in ground sections. Only one questionable lesion of this type was found in the unerupted teeth of sixty 18-day-old rats derived from nine litters. In 5,868 rats, from various maternal diets, the lesion was found on the left side in 208 rats, on the right side in 236 and bilaterally in seventy-eight.

30. The Qualitative and Quantitative Analysis of Bone "In Vivo" by Radiographic Technique. *W. A. Curby, Tufts College Dental School, Boston, Mass.* Radiographs of bone are made alongside an ivory reference step tablet. The ivory step tablet densities and bone densities are read on a Weston model 500 densimeter and plotted on special semilog graph paper against the corresponding bone or step tablet thickness. Variations in bone density caused by changes in deposition of original constituents show up as shifts up or down on the graph while variations in proportions of original constituents show up as variations in angles on the graph.

31. Oxidative Enzymes in Tooth Pulp, Gingiva and Sublingual Gland. *Bertram Eichel, USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.* Virtually no data exist which identify enzymes and metabolic pathways involved in energy metabolism in tissues of major interest to the dentist. Excellent biochemical methods for the quantitative detection of these essential cell constituents are available. The initial approach to this problem has been made, and several key oxidative enzyme systems have been identified quantitatively by spectrophotometric analysis in tooth pulp, gingiva, and sublingual gland tissue homogenates of normal New Zealand white and chinchilla grey adult female rabbits. In order to establish the relative metabolic levels of these components, comparative studies were run on liver, brain, retinal, and choroidal homogenates. The following components have been determined in each tissue: cytochrome oxidase, succinic dehydrogenase, catalase, and total protein. Each enzyme protein was readily detected in the tissue studied except glucose-6-PO_4 dehydrogenase in tooth pulp, sublingual gland, and brain. It is of definite interest that the dental tissues as well as the other tissues herein reported possess relatively high oxidative enzyme capacity. It is suggested that disturbances in these oxidation-reduction enzyme systems might be detected prior to visible changes associated with dental tissue pathology.

32. The pH and Lactate Content of Caries-Immune Dental Plaques. *H. R. Englander, W. J. Carter, and L. S. Fosdick, Dental Research Facility, United States Naval Training Center, Great Lakes, Ill., and Northwestern University Dental School, Chicago, Ill.* The pH and lactate content of caries-immune dental plaques were determined before and after a sugar rinse. It was found that none of the cases attained a pH of 5.5 or below after a thirty-second sugar rinse. By the use of the Hasselbach equation the free and neutralized lactic acid in the plaque was calculated. It was found that the amount of free lactic acid was always below the dangerous level, although considerable amounts of neutralized lactic acid were present. It was found that the buffering capacity of immune dental plaques was higher than that of caries-active cases.

33. Morphologic Effects of Irradiation on the Salivary Glands of Rats. *James A. English, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.* Salivary glands of rats were examined sixteen hours to 100 days following application of 200 KV x-ray radiation in doses of 1,000 and 1,500 r. A total of 162 rats were involved, seventy-two of them being used as littermate controls. Sufficient morphologic changes were observed in submaxillary salivary glands of animals sacrificed beyond the fifteen-day period to permit accurate separation of unmarked control and

irradiated tissues. The changes consisted primarily of alteration in size, shape, and staining characteristics of nuclei of acinar cells. Changes were observed in other salivary glands, but were not characteristic enough to serve as invariable means of identification.

34. Effect of 1, 2, 5, 6 Dibenzanthracene on Growth of Transplanted Tooth Germs. *Harold S. Fleming, Department of Pathology, Yale University School of Medicine, New Haven, Conn.* Growth of tooth germs, treated with 1, 2, 5, 6 dibenzanthracene and transplanted to selected locations in guinea pigs and dba mice, was altered. Where animals received brain transplants treated with 1, 2, 5, 6 dibenzanthracene, an initial toxic effect was often observed. In these instances one or two animals from a group died within twelve to twenty-four hours. This was not observed with controls nor with 20-methylcholanthrene–treated brain transplants. A toxic effect also seemed lacking when 1, 2, 5, 6 dibenzanthracene was transplanted alone to brains or with tooth germs to other locations. As with 20-methylcholanthrene, abundant keratin formations were produced. However, in such instances these formations appeared broader and assumed a deeper eosin stain. Keratin formations frequently seemed to arise directly from outer enamel epithelial cells and in a like way arose from certain areas of inner enamel epithelium. Nests of epithelial cells, presumably from the outer enamel epithelium, sometimes formed numerous small acinar structures intimately associated with the transplant. Other groups of cells, close to the transfer, often assumed bizarre sizes and presented numerous mitotic figures. Alterations in vascularity were consistently evident. Masses of red blood cells were usually seen in certain areas about these transplants, while associated blood vessels were both hyperemic and enlarged. For various periods (up to ninety days) 1, 2, 5, 6 dibenzanthracene appeared to exert a greater initial effect on tooth germ transplants than did 20-methylcholanthrene over similar periods.

35. The Effect of Hyaluronidase on Swelling and Trismus Resulting From the Removal of Impacted Mandibular Third Molars. *Philip T. Fleuchaus, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Twenty patients were chosen who had bilateral mandibular third molar impactions which were approximately the same in position. Only one side was operated upon at a time; the order of experimental and control side was also alternated. Local anesthesia was administered to block both the inferior alveolar and the long buccal nerve. The anesthetic for the control side consisted of 2 ml. of 2 per cent procaine with 1:50,000 epinephrine; for the experimental side, 30 turbidity reducing units of hyaluronidase were added to the anesthetic solution. Measurements of the thickness of the cheek and the amount of mouth opening were performed before each operation and each day following for five days by means of a caliper and Boley gauge. The operative procedure was identical in each case and no postoperative treatment either local or systemic was rendered. In comparing the amount of swelling in the cheek in the experimental and the control it was noted that the side treated with hyaluronidase was significantly less than the control side. Although the differences for the next three days were not significant, the experimental side tended to return to normal sooner. The amount of trismus resulting from the treated side does not show a significant difference from the control for the first day but the difference on the second and third postoperative days is highly significant. Although not markedly evident to the naked eye, hyaluronidase has reduced the trismus and lateral swelling of the cheek without any untoward effects and has provided greater comfort to the patient.

36. The Effect of Desalivation in Immature Hamsters on Their Dental Caries Susceptibility. *Sidney B. Finn and C. E. Klapper, University of*

Alabama School of Dentistry, Birmingham, Ala. It has been shown that the newly erupted hamster molar is most caries susceptible and that removal of hamster salivary glands increases caries susceptibility. On the basis of these observations, it was believed that removal of the salivary glands in young hamsters would result in maximum caries susceptibility. This hypothesis was tested by desalivating twenty hamsters at 27 to 34 days of age. All animals were fed a modified H. W. C. diet containing 60 per cent sucrose. Representative animals were sacrificed thirty-eight, forty-five, and fifty-two days after desalivation. Moderate to severe dental caries was noted after thirty-eight days and at the termination of the experiment (fifty-two days), destruction of the teeth was almost complete.

37. Cariogenic Effects of Skim Milk Powder Fractions and Heat-Processed Whey Powders. *J. E. Folk and F. J. McClure, National Institute of Dental Research, Bethesda, Md.* Chemical and biologic properties of skim milk powders are being studied in relation to their cariogenic effects in diets of experimental rats. Certain residues and extracts of autoclaved skim milk powders were found to be about equally effective in caries production. Dry whey powders, when present at a level of 25 per cent in rats' diets, produced smooth surface caries similar to that produced by skim milk powders. The dry whey powders also showed an increase in caries effect after a "dry autoclave" treatment.

38. Electrometric Studies of Human Gingiva in Pregnancy. *B. J. Gans, M. B. Engel, and N. R. Joseph, Loyola University Dental School, and Professional Colleges, University of Illinois, Chicago, Ill.* The gingival tissues were studied in women during pregnancy and after parturition. In about three-fourths of the cases the tissues appeared healthy. These patients were compared with a group of nonpregnant women whose gingivas were normal. The connective tissue changes in pregnancy were determined by measuring liquid junction potentials between sodium chloride solutions and the gingiva. A reference junction was made on the skin with 0.15M NaCl. Then 0.15M NaCl was instilled by a needle into the gingiva to give a base-line potential. This was usually around 0 mV. A dilution potential, E_d, was then measured by substituting 0.015M NaCl at the gingival junction. For the control group the mean E_d was 8.6 mV. The mean E_d for the pregnant group was 1.6 mV. After parturition the values rose again (mean $E_d = 5.4$ mV) approaching control levels. The differences between all groups were highly significant ($P < 0.001$). Through physicochemical theory it has been possible to relate the dilution potential to the density in equivalents of negatively charged colloid, x, of the connective tissue by the equation $E_d = -12.3 + 215\ x$. The potential drop in pregnancy is interpreted to reflect disaggregation of the mucoprotein ground substance of the gingival connective tissue. This is associated with uptake of water and changes in tissue electrolytes. The results demonstrate a sensitive response, often subclinical, of the gingiva to hormonal influences.

39. Sensory Responses Following Electronic Stimulation of Oral Tissues. Part II. The Medulla. *Joseph A. Gibilisco and Raymond Berlin, University of Alabama, School of Dentistry, Birmingham, Ala.* An understanding of the function of the trigeminal nerve is of importance to all aspects of dental practice. Unfortunately, our present knowledge of the detailed neurophysiology of the nerve is most incomplete. For these reasons a series of studies concerned with the function of its nerve fibers is being carried out. Fifteen cats have been used for this investigation. First, the oral tissues were stimulated, and the responses conducted through the Gasserian ganglion were recorded. The present investigation traces the pathway one step further.

Similar methods have been employed for electronically stimulating the teeth, the gingiva, and other areas innervated by the fifth nerve, but the responses have been taken from the descending root of the trigeminal nerve within the medulla. These studies have been limited to the right descending root. In each instance photographic records have been obtained with a cathode-ray oscilloscope. Histologic preparations of the medulla have verified the position of the electrode. Type A pain fibers have been found to predominate. Although there is evidence that proprioceptive impulses are carried in this tract, these observations are incomplete. We are currently attempting to verify neurophysiologically what has been established morphologically.

40. A Preliminary Report on the Effect of Sodium Fluoride on the Growth and Glycolysis of *Lactobacillus casei 4646*. *Robert E. Gillis, Department of Microbiology, Western Reserve University, Cleveland, Ohio.* For many years sodium fluoride has been known to inhibit anaerobic glycolysis through its effect on the enzyme enolase which catalyzes the reaction: 2 phosphoglyceric acid $\rightleftharpoons$ phosphoenolpyruvic acid. Experiments were devised to test the effect of increasing concentrations of this compound on the growth of *Lactobacillus casei 4646*. Growth was measured turbidimetrically in a Klett-Summerson colorimeter using a No. 66 filter. Inoculated fluoride-free media were used as controls. It was found that concentrations of sodium fluoride in excess of 0.02M markedly inhibited the growth of this organism. At 0.02M, growth was approximately 20 per cent as compared to growth in a fluoride-free medium. Nonproliferating cells of this organism grown in 0.02M sodium fluoride were tested for their ability to produce acid from glucose in the presence of a bicarbonate buffer at a pH of 6.8 and 0.025M sodium fluoride. In each experiment the cells were preincubated for twenty minutes in the presence of the inhibitor. Cells grown in fluoride-free medium were used as controls. The rate of glycolysis of the fluoride-grown cells was compared with that of the cells grown in the absence of fluoride, and it was found that while there was a marked initial inhibition of the fluoride-free cells, there was no inhibition of the fluoride-grown cells under these conditions. The following possibilities are suggested to explain this phenomenon: 1. The fluoride-grown cells have become impermeable to fluoride. 2. A fluoride-insensitive mechanism exists in the fluoride-grown cells. 3. These cells are metabolizing glucose to yield acid via a divergent pathway of glycolysis. Experiments are continuing to determine which of these alternatives is in operation.

41. A Provincial Survey of Caries Incidence. *R. M. Grainger and A. H. Sellers, Division of Medical Statistics, Ontario Department of Health, and D. B. W. Reid, Connaught Medical Research Laboratories, University of Toronto, Canada.* This survey will involve taking an annual stratified random sample of elementary school children of different ages. About 100 dentists will take part in examination of the children, so that the effect of individual biases will be greatly reduced. The children are selected by chance from school lists in sixty-four different areas of the Province. From the results of the survey, various indices of the caries prevalence may be calculated to furnish provincial estimates, and to draw comparisons between children under different dental health programs. In particular, the rate of accumulation of caries on smooth and pitted surfaces of the teeth will be estimated by regression after transformation to linearity, so that age- and sex-adjusted rates may be obtained. This will provide a satisfactory index of caries attack, provided changes tend to affect all ages similarly.

42. Transplantability of Experimentally Produced Tumors of the Salivary Glands of Mice. *Nicholas G. Grand and William H. Bauer, School of Dentistry, St. Louis University, St. Louis, Mo.* The present medical and

dental literature does not show any reports on experimentally produced transplantable tumors of the salivary glands. Five methylcholanthrene-induced parotid tumors of mice were found to be transplantable and were subjected to an extensive study. These tumors have been successfully transferred into mice (four in C strain and one in CFW strain mice) and are now in their twenty-second transfer. The tumors consisted of three adenoacanthomas and two undifferentiated carcinomas of the parotid. They grew very well in the axillary region of the mouse and 90 to 100 per cent of "tumor takes" were obtained. The transplants of these malignant neoplasms displayed the morphologic features of their original tumors. This was particularly the case in the transferred undifferentiated carcinomas while the transplanted adenoacanthomas revealed excessive hyperkeratinization and mucoid secretion. In few instances, metastases to the lung or regression of the tumors had occurred.

43. *Borrelia buccale:* Isolation, Pure Cultivation and Morphologic Characteristics. *Edward G. Hampp, National Institute of Dental Health, National Institute of Health, Bethesda, Md.* Of the bacteria comprising the oral flora, the spirochetes have presented the greatest difficulty in isolation and maintenance on artificial media. The smaller oral treponemes and *Borrelia vincentii* may now be isolated and cultivated routinely. However, up to this time, there is some doubt whether or not pure cultures of *Borrelia buccale* have ever been obtained on artificial medium. Thus far, four pure strains of *B. buccale* have been isolated and cultivated through repeated transfers. The method employed consists of the use of a modified Huntoon's hormone agar and a well technic previously described by the author for isolation of the smaller oral treponemes and *B. vincentii.* At the time of use, 10 ml. portions of the melted basic medium were enriched by the addition of 1 ml. of a 1 per cent solution of glutathione, 0.2 ml. of a 1 per cent solution of sodium pyruvate, 2 ml. of ascitic fluid and 1 ml. of ox serum concentrate. Subgingival scrapings from normal and diseased mouths were employed as sources of inocula. Primary well plates were incubated under strictly anaerobic conditions for two to three days, at which time the *B. buccale* had grown out into the medium adjacent to the well along with the other spirochetes. Subcultures were made from the outer area of the ring of spirochetal growth into the same medium as employed for primary plates. After establishing the presence of *B. buccale* and maintenance in mixed spirochetal culture for at least ten subcultures, isolation of this organism from the other oral spirochetes was accomplished by the use of shake tubes. The morphologic characteristics of the pure strains of *B. buccale* were investigated. Double contouring, spiral amplitude, abruptly tapered ends, and terminal filaments were studied by dark-field examination; flagella were demonstrated for the first time by electron microscopy. Certain of these pure cultures have been maintained for over eighteen months and through 100 transfers in the medium described.

44. The Reversal of the Caries-Potentiating Effect of EDTA on Rat Caries by a Salt Mixture. *M. Rachel Harris and Robert M. Stephan, National Institute of Dental Research, Bethesda, Md.* It has been found that the addition of EDTA (ethylene diamine tetra acetic acid) increased caries in rats fed on a fine particle diet 580 (Stephan and associates, *J. D. Res.* **31**: 475, 1952). The experiments reported here are based on diet 580 and a fine particle synthetic diet 593 (Stephan and Harris, *J. D. Res.* **32**: 687, 1953). The effects of adding 0.2 per cent EDTA to each of these diets and the counteracting effects of a salt mixture (Hubbel and associates, *J. Nutrition* **14**: 273, 1937) were studied. Eight groups of twelve Sprague-Dawley female rats were used, each group was given either diet 580 or 593 (1) alone, (2) with 0.2 per cent EDTA, (3)

with 4 per cent salts, or (4) with 0.2 per cent EDTA and 4 per cent salts. The addition of EDTA to the diets increased caries, whereas the addition of salts decreased caries and also overcame the caries-increasing effect of EDTA. The average number of carious lesions per animal on diet 580 was 5.18 without EDTA and 19.36 with EDTA, while on diet 593 the average was 7.25 without EDTA and 13.58 with EDTA. With the addition of salts the average number of carious lesions per animal on diet 580 was 1.36 without EDTA and 1.46 with EDTA, while on diet 593 the average was 0.0 without EDTA and 7.46 with EDTA.

45. Tracer Study of the Effect of Acute and Chronic Exposure to Sodium Fluoride on the Thyroid Iodine Metabolism of Rats. *Norman O. Harris, USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.* The addition of small amounts of fluorine to municipal water supplies as a possible means of mass dental caries control, although generally favorable from a strictly dental viewpoint, has precipitated considerable controversy due to the possibility of adverse systemic reactions. It is probably in the field of thyroid pathology that the greatest number of disputes have arisen on the use of this trace element. Both inhibitory and synergistic effects have been alleged. A tracer study was therefore instituted to test the effect of acute and chronic administration of fluoride on the thyroid iodine-131 metabolism of rats. Thyroid uptake, transport from the acinar cells to colloid, and blood plasma iodine levels were studied. The results of this experiment indicated that fluorine had no discernible effect upon thyroid iodine metabolism.

46. Pilot Studies of Three Topical Fluoride Application Procedures. *Roland R. Hawes, Solveig Sannes, and Finn Brudevold, Eastman Dental Dispensary, Rochester, N. Y.* A 15 per cent solution of sodium monofluorophosphate was applied unilaterally once every four months for one year to the teeth of 150 boys and girls whose average age was 12.1 years. At the end of the year there were 20 per cent fewer new decayed and filled surfaces among the treated teeth continuously present and 30 per cent fewer new decayed and filled surfaces among teeth erupted during the year in which there were one or two treatments. A 10 per cent solution of potassium fluoride was applied unilaterally in the first and seventh months of another one-year study in 117 boys and girls whose average age was 10.7 years. There were 30 per cent fewer new decayed and filled surfaces among the treated teeth present for the full year, but less than a 20 per cent reduction occurred in the erupted teeth. In a third study, teeth on one side of each child's mouth were treated four times in the first month of the study while teeth on the opposite side were treated once every four months for the entire three years of the study. A 1 per cent sodium fluoride solution was used. Forty-six boys and girls were available for the whole three-year study. Comparison of the caries increments indicate a possible cumulative benefit to teeth present throughout the study and treated every four months and a 30 per cent reduction in decayed and filled surfaces in newly erupting teeth by this procedure.

47. In Vitro Studies of the Uptake of Fluoride on Tooth Surfaces From 1 PPM Radioactive Fluoride Solutions. *John W Hein, John Bonner, Finn Brudevold, and Harold C. Hodge, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Radioactive fluorine prepared by proton bombardment of frozen samples of water enriched in O^{18} was added to 1 ppm F as NaF solutions. Extracted teeth of known age and history were coated with inlay wax except for one enamel surface. Impressions of these teeth were made with compound on $\frac{1}{16}$ inch aluminum plates designed to fit the Geiger

counter so that the exposed enamel surface could be repositioned over a 3/32 inch hole in the plate through which repeated surface counts of same tooth area could be taken. It was found that freshly extracted teeth cleaned with brush and water picked up relatively little fluoride. Repeated pumicing of teeth gave progressively greater pick-up, indicating that the surface of the tooth is less reactive than deeper layers. Vigorous mechanical brushing with water removed some of the fluoride, but even less than five seconds of pumicing removed nearly all the fluoride. Thirty seconds' polishing with a calcium phosphate toothpaste removed nearly one-half of the fluoride. Studies with fluoride solutions of varying pH showed that high pH solutions gave little pick-up, low pH solutions increased pick-up. A wide difference in reactivity to fluoride was noted between different teeth and between areas on same tooth. Pick-up in white spots on enamel was much higher than in the rest of the enamel surface.

48. The Evanston Dental Caries Study. XII. A Survey of Lactobacillus Counts With Reference to Untreated Carious Surfaces Before and After Exposure to Fluoridated Water. *I. N. Hill, Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* It is the purpose of this report to attempt to draw some conclusions from the data gathered in the Evanston Dental Caries Study regarding the relation of lactobacillus counts to dental caries prevalence rates. Cases were grouped according to lactobacillus count; low division comprised counts ranging from 0 to 9,999; moderate division cases were those with counts of 10,000 to 49,999 and high division cases were those with counts of 50,000 and over. Negative lactobacillus count cases included in the low division were separated and tabulations were made of them. From 1946 through 1952, 1,991 cases were reported. The percentage of low division cases with negative lactobacillus counts increased from 43.75 per cent for the 6- to 8-year-old group in 1946 to 63.23 per cent in 1951. In the older age group (12- to 14-year-old children) the percentage of low division cases with negative lactobacillus counts rose from 39.13 per cent in 1946 to 42.63 per cent in 1952. Fluorine was added to the Evanston water supply in 1947. For the older age group the number of untreated carious surfaces found in the low lactobacillus count division was reduced 17.55 per cent from 1946 through 1952. In the moderate lactobacillus division the untreated carious surface rate was reduced 19.21 per cent and in the high lactobacillus count division a reduction of 38.17 per cent was noted.

49. The Evanston Dental Caries Study. XIV. Effect of Sodium Fluoride in Communal Water Supply on Caries Rates of 6-, 7- and 8-Year-Old Children. *I. N. Hill, J. R. Blayney, and W. Wolf, Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* A comparison of DMF caries rates of Evanston school children examined in 1946 is made with those of children examined in 1953. The children examined in 1953 were exposed to the fluoridated water seventy-one to eighty-two months. The combined 6- to 8-year-old children had a permanent tooth DMF rate of 149.76 per 100 children in 1946 and 65.82 in 1953. This is a difference of 60.38 per cent. The differences in rates associated with the permanent teeth were considered statistically significant. In considering the deciduous tooth caries rates it is interesting to note that these 6-year-old children were born during the same year that fluorine was added to the water and that their caries reduction from the statistical standpoint was considered borderline. The 7-year-old children experience about one-half the reduction of the 6-year-old children, and the 8-year-old children had a slight increase in the caries rate when expressed as per 100 children.

50. Progress Report on the Breeding of Caries-immune and Caries-susceptible Strains of Syrian Hamster. *Harold C. Hodge, Erling Johansen, John W. Hein, and Elliott A. Maynard, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* From the third generation of cross-bred hamsters previously studied (*J. D. Res.* **32**: 654, 1953) for a genetic pattern of caries susceptibility or caries resistance, two susceptible lines were started for inbreeding. Both lines have been carried forward to the third generation of inbreeding (sixth generation of study). Of the two lines of resistant animals selected at the same time, one has been successfully carried forward to the sixth generation, the last two being inbreedings. Because of the difficulty with reproduction in the animals raised on the original diets—a difficulty since overcome—additional lines have been started. These were chosen from a caries screening of 128 males and 116 females from our stock colony. A resistant line was begun from two brothers and two sisters with zero scores. Four males and four females from three litters were selected for three additional susceptible lines. These lines are now in the third generation of inbreeding.

51. The Pathological Characteristics of Experimental Fusospirochetal Infections. *H. A. Hunter, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* Evidence concerning the production of toxic materials in tissues which are the seat of fusospirochetal infections is inconclusive. To further this subject, twelve cats, twenty-two guinea pigs, and twelve rabbits were inoculated subgingivally, subcutaneously, and intravenously, respectively, with pooled human gingival scrapings or guinea pig fusospirochaetal exudate and Seitz filtrates prepared from scrapings or exudate. Filtrate of scrapings or exudate did not produce demonstrable lesions in any instance. The 0.5 ml. intragingival inoculation of scrapings in cats produced local acute abscesses in two of four animals. These discharged spontaneously and healed. The 0.1 ml. subcutaneous guinea pig inoculations produced either local acute abscesses that healed or a spreading cellulitis that ultimately killed the animal. Five of six rabbits injected intravenously with 0.1 ml. of exudate died of intercurrent bronchopneumonia. Lung and liver abscesses were found in three of them.

52. Response of Human Pulp to Paraformaldehyde and Cavity Preparation. *Verda Elizabeth James and J. P. Weinmann, Departments of Dental Histology and Oral Pathology, University of Illinois, College of Dentistry, Chicago, Ill.* This study was based on the histologic investigation of twenty-one human teeth in which cavities were prepared by bur or by airbrasive techniques. Two and one-half per cent paraformaldehyde in an aquadont base was placed in the cavities and sealed with zinc oxyphosphate cement. The teeth were extracted after periods of time ranging from three to ninety-six days. Nonexperimental areas in the pulps of the experimental teeth and pulps of unoperated teeth from the same group of patients were used as controls. No significant differences in the reactions to cavity preparation by the bur technique or by the airbrasive technique were noted. Constant findings consisted of a breakdown of the dentinopulpal membrane, hemorrhage, and severe alterations in the odontoblasts. The pulpal alterations were confined to the area below the cavity, and were characterized by the degeneration and necrosis of many of the odontoblasts and cessation of dentin formation. The layer of Weil was invaded by fibroblasts which differentiated into preodontoblasts and young odontoblasts. A number of them also underwent degeneration and necrosis. Gradually, however, an increasing proportion of the young cells survived and differentiated sufficiently to produce a secondary

dentin which was poorly calcified and showed an irregular course of the dentinal tubules. Macrophages were observed as early as the first week and persisted through the ninety-six-day survival period. In contrast, the neutrophilic leukocytes first appeared at the end of two weeks and were soon replaced by lymphocytes which were still present in the ninety-six-day specimen.

53. Attempts to Produce Dental Caries in the Syrian Hamster by Controlled Topical Applications of Sucrose and Acids. *Erling Johansen, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The observation that hamsters sometimes develop caries lesions in a matter of days has invited a new approach for studying the caries process in animals and for testing various theories of dental caries. A series of experiments has been carried out during the last two and one-half years attempting to reproduce on susceptible areas of live hamster molars in situ conditions thought necessary for caries development. The device previously described (*J. D. Res.* **31**: 361, 1952) was originally designed for these studies allowing simultaneous mechanical holding and immobilization of animals in the focusing range of a microscope. Using this technique, a variety of substances—high sucrose diets, powdered sucrose, sucrose solutions, carious material from lesions in other hamsters, bread, acids, and acetate buffer—has been applied topically at various time intervals to selected quadrants, with opposite quadrants serving as control areas. In certain studies lasting for a few days some animals have been fed by stomach tube. Others were allowed to eat a starch-containing stock diet during the experiment while sucrose was applied to certain molars every twenty to thirty minutes, ten to twenty times a day, six days a week totaling up to 950 applications in eighty-three days. Gold bands were also placed on a number of teeth to assure debris accumulations and to retain the applied sugar. In spite of all the substrate furnished and the excellent opportunities for the microorganisms to lodge on tooth surfaces under the conditions of some of these experiments, the lesions that developed were few and extremely small. As determined by gross examination (36×), all lesions, with one exception, were located in the experimental quadrant, indicating the importance of sucrose in the development of these lesions. Repeated controlled applications (up to 150 times) of microquantities of lactic acid (pH 3) and acetic acid sodium acetate buffer (pH 4) to susceptible tooth surfaces did not produce grossly detectable changes in the enamel over a two-week period. By the application of more concentrated acid solutions and with longer times of exposure, decalcification of the live tooth was observed. By this means an effort is now being made to break down the resistance of the tooth.

54. Hexokinase Activity in Human Saliva. *R. T. Jones, Jr., and V. F. Lisanti, Tufts College Dental School, Boston, Mass.* This enzyme was determined by the measurement of the rate of conversion of available reducing sugar to the phosphorylated form in the presence of A.T.P. and magnesium ions. One milliliter of saliva was incubated at 37° C. with 1 ml. of a reaction mixture containing 7×10^{-3} M sugar, 7×10^{-3} M A.T.P., 1×10^{-2} M $MgCl_2$, and 6×10^{-2} M "Tris" buffer at pH 8. Final reducing sugar concentrations were measured in a photoelectric colorimeter at 540μ by the Nelson method. A 3.3×10^{-3} dilution of a partially purified hexokinase preparation incubated for two to thirty minutes showed a disappearance of 6 to 72 per cent of the initial glucose concentration, respectively: a 6.7×10^{-4} dilution after forty minutes' incubation showed 49 per cent disappearance of available glucose. Whole saliva incubated for five to sixty minutes gave an average glucose conversion of 11 to 56 per cent. These preliminary results indicate that

hexokinase is present in human saliva. Studies are in progress to establish the possible relationship of hexokinase activity of saliva to normal and pathologic oral conditions.

55. A Study of Efocaine. *Paul E. Jurgens, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Efocaine has been offered to the profession as an injectable, long-lasting local anesthetic solution, to counteract postoperative pain. The manufacturer states that it will safely produce anesthesia for twelve to fourteen days. Its constituents are procaine 1 per cent, procaine hydrochloride 0.25 per cent, butyl para-aminobenzoate 5 per cent, polyethylene glycol 300 2 per cent, propylene glycol 78 per cent, sodium metabisulfite 0.1 per cent, phenyl mercuric borate 1:25,000, and water. Efocaine and each of its constituents were injected intravenously, intramuscularly, intraperitoneally, intraspinally, and subcutaneously in fifty hamsters, six rabbits, and four dogs. The animals were sacrificed after varying intervals and the tissues examined microscopically. In all areas where injected, Efocaine produced extreme and complete necrosis. When injected superficially, sloughing and ulceration occurred. Propylene glycol alone produced a similar reaction but not as severe as the mixture. Polyethylene glycol 300 produced somewhat less necrosis than propylene glycol. When given intravenously or intraperitoneally in large enough doses, Efocaine produced opisthotonus, cyanosis, loss of equilibrium, respiratory arrest, and usually immediate death. Propylene glycol appears to be the agent responsible for the toxic effects. This study indicates that in its present form Efocaine is a dangerous, toxic drug.

56. Isolation and Cultivation of Filamentous Bacteria From the Human Mouth. *George J. Kasai, the Walter G. Zoller Memorial Dental Clinic and the Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.* Several different media were compared for isolation of the filamentous oral bacteria. The most favorable result, from the standpoint of number of colonies of filamentous bacteria, was secured with a medium consisting of tryptose, soluble starch, yeast extract, and agar (TSY). Other plating media used were brain-heart infusion agar (BH), BH supplemented with thioglycollate and serum albumin (BHTS), blood agar or its many modifications, and several meat or yeast infusion media. TSY supported the growth of six to eight times as many colonies of filamentous bacteria, and BHTS about twice as many as the BH medium. Other media were not superior to the BH medium. All media were adjusted to pH 6.8. Specimens were taken by means of a sterile dental scaler from teeth and suspended in sterile buffered saline solution. These suspensions were broken up and streaked on the agar media within thirty minutes after collection and incubated anaerobically with CO_2 at 37° C. After five to seven days' incubation, these plates were examined for the presence of filamentous forms which could be easily identified by four or more different morphologic types. These were further isolated and purified by restreaking on agar through several transfers. The colonial morphology remained unchanged and the organisms continued to require anaerobic or microaerophilic conditions. Gram stains revealed many of these organisms to be gram positive or gram variable while a few were gram negative. The majority of them tended to break up into shorter forms with a few long filaments scattered here and there.

57. The Electron Microscopy of Caries in Primary Teeth. *James J. Kennedy, National Institute of Dental Research, Bethesda, Md.* Structural alterations in carious enamel and dentin of primary teeth have been observed in thin sections with the electron microscope. Only portions of these tissues

which could be cut directly without artificial demineralization were utilized. Small chips, approximately 0.25 mm. in diameter, were dissected from carious lesions of primary teeth, dehydrated in cellosolve, and embedded in plastic. Specimens were cut with both the Spencer rotary microtome using the thermal expansion technique and the Minot International microtome with mechanical advance. Observations were made on the basis of location of bacteria and the pathways through which they penetrate, sequence of breakdown of tissue components in both bacterial invaded areas and those that are bacteria free, degree of mineralization, and appearance of the organic matrix. Typical electron micrographs are presented and compared with those made from similarly prepared sections of carious enamel and dentin of permanent teeth.

58. Effect of Inhalation of Airabrasive Powder. *Donald A. Kerr, Sigurd Ramfjord, and Greta M. Ramfjord. University of Michigan, School of Dentistry, Ann Arbor, Mich.* A method was developed for exposing animals to an atmosphere containing adequate airabrasive dust to ensure inhalation. Rabbits, guinea pigs, and monkeys were exposed to new and used airabrasive powder for one or six hours per day. The time of exposure was from thirteen to 222 days, and the animals were sacrificed from fifteen to 648 days after the initial exposure. The characteristic necropsy findings were patchy atelectasis and emphysema. Mild patchy fibrosis was associated with airabrasive powder in the lungs. Free particles of dust were found in bronchi and in the peribronchial alveoli. Phagocytized particles were observed in perivascular and peribronchial tissues as well as in peribronchial lymph nodes. The effect varied in different species and also in animals of the same species.

59. Influence of Bacterial and Chemical Agents on Connective Tissues. *Grace Kimball, Institute of Pathology, Western Reserve University, Cleveland, Ohio.* A method is described for the utilization of the hamster pouch for the determination of the effect of various agents on oral tissues. Early damage was detected by cytochemical methods. Among the agents tested were cultures and filtrates of common mouth organisms, hyaluronidase, and histamine. Of these, histamine and alpha streptococci appear to be the most effective.

60. The Influence of Selected Sugars on the Dental Caries Susceptibility of Desalivated Hamsters. *C. E. Klapper and J. F. Volker, University of Alabama School of Dentistry, Birmingham, Ala.* It has been demonstrated in these laboratories that moderate to severe dental caries can be produced in mature young hamsters within thirty-five days after they have been placed on a cariogenic diet. These observations have been extended to include the relative abilities of selected sugars to enhance or retard caries susceptibility. Twenty-seven animals were desalivated at 39 days of age. At the forty-sixth day, when they had recovered from the operation, they were divided into Groups A, B, and C. Group A animals received a modified H. W. C. diet containing 60 per cent carbohydrates, 30 per cent of which was whole wheat flour and 30 per cent sucrose. Groups B and C animals received identical diets except that the sucrose fraction was completely replaced by fructose and sorbitol, respectively. Thirty-five days later the experiment was terminated and the molar teeth were examined for caries. All the animals showed some degree of caries. The extent of the caries in Group A (sucrose) was approximately twice that of Group B (fructose) and Group C (sorbitol). The findings in Group C suggest that under conditions favoring prolonged oral retention, sorbitol may be cariogenic.

61. Further Observations on the Effect of Tobacco Smoke on Epithelial Tissues of Vitamin-Deficient Mice. *Seymour J. Kreshover, School*

of Dentistry, Medical College of Virginia, Richmond, Va. A previous study indicated that whole tobacco smoke applications to the lips of castrate and noncastrate mice maintained on normal and vitamin A and B complex–deficient diets caused little response, whereas similarly treated ears showed appreciable change. Although the minimal lip reaction was attributed to the protective effect of the tongue and saliva in removing deposited tars, the ear findings suggested that B complex deficiency increases tissue susceptibility and gonadectomy establishes a degree of resistance. In order to investigate further the question of B deficiency as a predisposing factor to increased tissue susceptibility, 201 additional animals were studied. By means of a specially designed apparatus, smoke applications were made to the ears of 172 noncastrate animals rendered deficient in various components of the B complex (riboflavin, pyridoxine, pantothenic acid, thiamine, biotin, and niacin). Each mouse received a daily application of equal volume and exposure time ranging up to forty days. Another group of twenty-one animals served as controls to rule out any significant influence of pressure and suction alone, incident to the use of the smoking machine. Gross and microscopic findings in the experimental groups showed that riboflavin and pyridoxine deficiencies are the fractions of B complex most responsible for tissue susceptibility (characterized by hyperkeratinization, hyperplasia, acanthosis, dyskeratosis, inflammation, and fibrosis). Appreciably less severe changes were noted in the pantothenic acid- and thiamine-depleted animals, and practically none in the niacin and biotin groups. When the effects of gonadectomy were re-evaluated in eight riboflavin-deficient animals, it was found that the degree of tissue response was decreased, although less so than previously reported.

62. Reaction of Gingival Tissue to Abrasive Injury. *John A. Kollar, Jr., and Frank M. Wentz, Loyola University School of Dentistry, Chicago, Ill.* Healing or tissue repair has been previously described as following or accompanying inflammation. Using six selected individuals free from papillary or marginal gingivitis, the effect of injury to gingival tissue was studied. The epithelium on papillary and attached gingival areas of the maxillary and mandibular canines and premolars was abraded off, using a coarse grit, aluminum oxide powder and an Airdent machine. Biopsies were taken: 1. before injury, 2. immediately after injury, and at six-hour intervals for twenty-four hours. A final specimen was taken at forty-eight hours. Specimens were fixed in Zenker formalin and studied histologically. Zero-hour specimens showed, clinically, a freely bleeding surface and, microscopically, complete destruction of surface epithelium and ridges as well as penetration of crystals into connective tissue. Six-hour specimens showed clinically and microscopically a surface clot impregnated with crystals. No inflammatory reaction was observed in the connective tissue, even around imbedded crystals. Twelve-hour specimens showed beginning proliferation of epithelium under the coagulated necrotic surface but no inflammatory reaction in the connective tissue. Eighteen-hour specimens showed almost complete coverage of wound surface, imbedded crystals still present but no inflammation. Twenty-four-hour specimens showed complete epithelization and beginning ridge formation. Numerous mitotic figures were found close to the basal cell layer; the necrotic layer was beginning to disintegrate. The forty-eight-hour specimens showed little indication of previous injury, and no evidence of inflammation was observed.

63. The Action of Oral Microorganisms on the Chondroitin Sulfate of Dentin. *W. A. Konetzka, M. J. Pelczar, Jr., and G. W. Burnett, Department of Bacteriology, University of Maryland, College Park, Md.* Since bacterial chondroitin sulfatase has been postulated as being involved in the

carious process, studies have been undertaken to determine the action of oral microorganisms on chondroitin sulfate, a polysaccharide present in dentin. Of numerous representative oral bacteria examined, only a few species represented by gram-negative, nonsporeforming, facultative anaerobic rods were capable of producing an acid reaction in a medium containing chondroitin sulfate as the main energy source, indicating possible degradation of the polysaccharide. Resting cells of one of these bacteria, *Proteus vulgaris,* were employed to study the factors affecting the release of reducing sugars, sulfate ions, and ammonia during the hydrolysis of chondroitin sulfate and to study the conditions necessary for the production of the enzymes by this bacterium. The enzymes could not be demonstrated in resting cells of *P. vulgaris* when the organism was grown in the absence of chondroitin sulfate, but once exposed to the substrate in concentrations as low as 250 μg per milliliter, the cells continued to be active after several transfers in a medium lacking chondroitin sulfate, suggesting selection of the active cells rather than adaptation. Attempts were also made to select cells by growth in media containing intact and powdered teeth. Resting cells of mixed cultures obtained by the incubation of saliva or material from the carious lesion released reducing sugars and ammonia from chondroitin sulfate, but free sulfate ions could not be detected. Attempts were made to isolate the active organisms from the mixed culture by use of enrichment technics.

64. An Investigation to Determine the Effects of Starch Sponge Implanted in Bone. *Leo Korchin, School of Dentistry, Georgetown University, Washington, D. C.* Starch sponge, an absorbable hemostatic agent, is made by freezing a starch paste, after which it is thawed, dried, and sterilized. Data on absorption and effects of this material on soft tissue are already available. The purpose of this investigation was to determine: (1) the biologic absorbability of starch sponge in bone; (2) the histologic response that might be provoked by implantation of starch sponge in bone; (3) the effect upon healing following implantation of starch sponge in the bone; (4) the incidence of alveolar osteitis following implantation of starch sponge in the tooth extraction socket. The first three phases of this investigation were studied in the dog, the fourth in human beings. Implantation of starch sponge was done in crest of ilia and maxillary tooth sockets in dogs for comparative study of absorption and histologic response. The data for the last phase were collected on human beings in a dental clinic. It was found that starch sponge is absorbed at approximately the same rate as the blood clot, leaving residual granules which were present at twenty-eight days. Its use in the dog tooth socket is accompanied by an acute inflammatory reaction with resultant delay in healing. When used in the human tooth socket the incidence of alveolar osteitis did not increase significantly, but healing appeared to be delayed.

65. Suggested Standards for Clinical Evaluation of Proposed Caries Control Agents. *Benton Kutler, University of Nebraska College of Medicine, Omaha, Neb., and United States Army Hospital Dental Clinic, Fort Jackson, S. C.* The recent history of clinical studies of caries control agents emphasizes the need for standardization of clinical research on which valid conclusions may be based. The following are suggested standards: (1) Randomization in selecting control and experimental patients. (2) Exact anatomic recording of all restorations and all lesions, as well as using different coloring to denote type of restorative material and to differentiate lesions of first and second examinations. (3) All lesions recorded—carious or precarious—and extent noted. (4) Thoroughness in use or application of agent (but isolated from the nontreated quadrants if the controls are in the same individual). (5) Number

of auxiliary agents used kept to a minimum and duly noted. (6) Complete thoroughness of examination. (7) First and second examinations of individual made by same examiner. (8) Examiner must have no conscious or unintentional knowledge as to whether he is examining control or experimental. Having approximately equal numbers of each serves to help eliminate unintentional or subconscious knowledge. (9) Conclusions as to the caries-affecting properties of agent used should be presented in terms of number of individuals benefited, harmed, or not affected. There should be no lumping together of total caries attack rates. (10) Regardless of location all carious lesions should be utilized as indicators of attack rates, rather than using only carious surfaces or teeth. None of these standards should be sacrificed to attain a larger sample size.

66. The Metabolism of Bone From Normal and Parathyroid Extract-Treated Rabbits. *D. M. Laskin and M. B. Engel, University of Illinois College of Dentistry, Chicago, Ill.* The metabolism of epiphyseal bone slices from young rabbits was studied using the Warburg respirometer. The influence of parathyroid extract was tested in one group receiving 1,000 units in forty-two hours. The rate of endogenous anaerobic glycolysis in control and treated animals did not differ significantly. Endogenous respiration (Q_{O_2} [DNA]) in untreated animals was 57 ± 1 μl oxygen per hour per milligram desoxyribose nucleic acid. Respiration was depressed in slices from animals receiving hormone and the Q_{O_2} (DNA) was 33 ± 2.7. In both groups the addition of glucose, pyruvate, citrate, fumarate, alpha-ketoglutarate, malate, and methylene blue did not affect respiration. Succinate stimulated oxygen uptake in all animals. This effect was inhibited by malonate, confirming the presence of succinic dehydrogenase. Dehydrogenase activity was measured by determining the reduction of triphenyltetrazolium chloride in the presence of succinate. This was found to be decreased by one-half in the hormone-treated animals. The resorption of bone following administration of parathyroid extract may be related to this change in the activity of the intracellular dehydrogenases. This event could lead to an accumulation of some of the intermediates of carbohydrate metabolism, particularly the salts of the di- and tricarboxylic acids. These substances could act at physiologic pH to combine with insoluble minerals of the bone matrix and of the cells by cationic exchange. This would result in the dissolution of the structure and enhance the transfer of mineral salts across cell membranes.

67. The in Vitro Cultivation of Rat Molar Tooth Germs on the Clot. *William Lefkowitz and Patricia Swayne, College of Dentistry, The Ohio State University, Columbus, Ohio.* The Carrel flask technic has been successfully utilized for in vitro culture of tooth germs in the clot. The results showed approximately two days' development and growth. The tooth germs lost their normal configuration and became flat after sixteen days of cultivation. On the clot cultivation, using the same media as in the previous report (*J. D. Res.* **33**: 181, 189, 1954), showed the same degree of development, growth, and survival. The cultures maintained their normal gross anatomy.

68. Storage of Fluorine in the Developing Rat Embryo. *David Lehman and Joseph C. Muhler, Department of Chemistry and School of Dentistry, Indiana University, Bloomington, Ind.* It is important to know if fluorine is transferred through the placenta and stored in the skeleton and teeth in women using a fluoridated water at about 1 ppm of fluorine. Previous experience gained by observing the degree of bleaching of rats' incisors has indicated that such a transfer is possible, but only at relatively high, and thus

unphysiologic, conditions. In this experiment, three groups of rats were fed fluoride supplements of varying concentrations, and after mating and birth of the young, the newborn were immediately analyzed for fluorine. Six groups of female rats were fed 50, 25, and 0.5 ppm F (as NaF) in the drinking water, the supplements were continued at this level throughout pregnancy. The results show a tendency toward a direct proportionality between the amount of fluorine ingested by the pregnant animal and the amount found in her newborn.

69. Effect of Beryllium Upon Hamster Caries. *H. M. Leicester, P. R. Thomassen, and L. W. Nicholas, College of Physicians and Surgeons, San Francisco, Calif.* Thirty-four male hamsters were placed upon a diet containing .02 per cent $BeCl_2$ in food and drinking water from date of conception until 24 days of post-partum life. They were then fed a cariogenic diet for 100 days. A control group of forty-five males was run concurrently. At the end of this period, both groups were sacrificed. The jaws were removed from each animal and stripped of soft tissue. The teeth were then scored for caries under a 20-power binocular microscope. The controls had a mean occlusal score of 4.51 ± 4.78. Five animals were free of caries. The experimental group had a mean occlusal score of 1.02 ± 2.04. Eighteen hamsters were free of caries. Computation of the "t" value for these animals gave a value of 3.986. This places the "probability" well below the 0.01 level of significance.

70. The Effect of Silicones and Lactones on Synthetic Calculus Formation. *S. Wah Leung, University of Pittsburgh School of Dentistry, Pittsburgh, Pa.* The effect of silicones on calculus formation was tested, under the in vitro conditions previously described, by coating the teeth with a single application of one of five silicones, before subjecting them to four to six weeks of intermittent immersion in saliva. The results indicate that such a coating was ineffective in preventing the deposition of synthetic calculus on these teeth. Three sugar lactones, glucono-, galactono-, and glucurono-lactone, were also tested by adding the lactones daily to fresh saliva to a final concentration of 0.5 per cent. The teeth, untreated, were subjected to the action of the lactone-saliva for periods varying from four to six weeks. The lactones, used in this manner, almost completely inhibited deposit formation on the teeth. However, some of the teeth, especially those in the glucono-lactone, showed a whitening characteristic of a mild decalcification. Coating the teeth with one of the silicones before subjecting them to the lactone-saliva mixture prevented the decalcifying action of the lactone without any apparent decrease in the calculus-inhibiting property of the lactone.

71. The Effect of Fluorine on the Deposition of Radiocalcium in the Rat. *R. C. Likins, I. Zipkin, A. C. Steere, and F. J. McClure, National Institute of Dental Research, Bethesda, Md.* Four littermates from each of eight litters of weanling female rats were distributed equally among four experimental groups. The animals of two groups received 10.0 mg. of fluorine (NaF) by stomach tube in twenty equally divided portions administered five days a week for four weeks; those of the remaining groups served as controls and were intubated with distilled water. All animals received 1 μc of carrier-free $Ca^{45}Cl_2$ by intraperitoneal injection thirty minutes after each intubation. On the thirtieth day, the members of one fluoride and one control group were sacrificed; the remaining groups were continued without further treatment and sacrificed on the sixtieth day. The rats were then autoclaved and the femurs, mandibles, and teeth removed for analysis. At the end of the first thirty days the bone ash of the test animals contained approximately thirty times more fluorine (per cent) than the controls, whereas the fluorine content

of the incisor and molar dentin was approximately fifteen times that of the controls. During the next thirty days there was a significant ($p < 0.05$) loss of total fluorine from the test femurs, mandibles, and incisor dentin, but not from the molar dentin. At thirty days the specific activity of Ca^{45} was in the following order: femur, incisor enamel, and dentin > mandible molar dentin > molar enamel. The data further indicate that the rate of loss of radiocalcium from the tissues of the test animals is somewhat greater than from those of the controls, with the exception of the molar enamel.

72. Comparative Study of the Enamel Decalcifying Ability of Different Acids. *Marguerite F. Little, Michael G. Buonacore, and Biruta Sommer, Eastman Dental Dispensary, Rochester, N. Y.* The ability of nine organic acids to decalcify enamel was studied. The action of 20 c.c. of each acid solution on 100 mg. of powdered enamel was compared at 0.1N concentrations and at buffered pH of 4 over a thirty-minute period. With pyruvic, tartaric, acetic, aspartic, and glutamic acids at 0.1N, the amount of enamel dissolved was directly related to the degree of acidity of the acid solution. Malic, formic, citric, and lactic acids dissolved, respectively, 45.7, 57.5, 39.5, and 60.7 mg. of enamel. Phosphorus determinations were made on the acid solutions after reaction. Results indicate that the gravimetric values given previously are correct and are not false values due to precipitation of insoluble salts. Results with malic and citric acids are out of line, compared with the weaker acids, formic and lactic. Retesting the former acids on molarity basis gave results of 73 and 82 mg. of enamel dissolved, respectively. This is consistent with the strength of these acids. Total acidity is, therefore, not a valid basis for comparison of acid action. On the basis of the milligrams of enamel dissolved at constant pH 4, the acids are divided into four groups: Group I acetic, lactic, 33.7 – 35.2; Group II citric, formic, tartaric, 29.1 – 31.5; Group III pyruvic, malic, 24.5 – 25.3; Group IV aspartic, 12.6. The value obtained with aspartic acid suggests that enamel solubility is effected by factors other than hydrogen ion concentration. If acids showing similar behavior exist in a carious area or in a plaque, then we have further reason to question the validity of plaque pH as a measure of the potentiality for enamel destruction without a knowledge of the kind of acid involved.

73. Acid Formation in the Mouth After the Ingestion of Different Carbohydrate Foods. *Thomas G. Ludwig and Basil G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* For five test subjects, a study was made of the production of acids in the dental plaque after nine different carbohydrate foods had been eaten. Acid formation was determined by measuring the pH of the plaque using a one-drop glass electrode attached to a Beckman potentiometer. Since it is difficult to make these measurements accurately, as a confirmatory test, the production of lactic acid in the saliva after the consumption of these foods was determined. For this purpose the method of Barker and Summerson (1942) was used. It was found that some of the foods tested stimulated the production of greater quantities of acid in the plaque and in the saliva for longer periods of time than did others. Fig-containing cookies, toffees, and bread and jam produced the greatest quantities of acid, while fried potatoes, ice cream, apples, and various sweetened beverages produced relatively smaller quantities. From these findings it was concluded that some carbohydrate foods may be more likely to produce caries than other similar foods.

74. The Production of Fusospirochetal Infections in Guinea Pigs With Recombined Pure Cultures. *John B. Macdonald, Ruth M. Sutton, and M. L. Knoll, Division of Dental Research, Faculty of Dentistry, University of*

Toronto, Toronto, Canada. Nine attempts were made to recombine sixteen distinct bacterial strains and a culture of spirochetes isolated from an experimental guinea pig fusospirochetal exudate. Each pure culture was inoculated in turn onto an enriched blood agar plate as a single streak from the edge of the plate into a drop of broth in the center of the plate. Each streak ultimately appeared as the spoke of a wheel with the central drop in which all cultures were merged forming the hub. Spirochetes were inoculated into the central drop without being streaked. Growth in three of the nine trials was considered satisfactory. All strains grew along the whole length of the streak in two instances and all but one in a third. Spirochetes grew in all three. In these three trials, the mixed growth in the central drop was subcultured once as unstreaked drops and the resultant growth in each trial was emulsified and inoculated into four guinea pigs. Mixed growth of unpurified exudate inoculated into four guinea pigs served as controls. Exudate from appropriate lesions was aspirated and passed to fresh guinea pigs, a procedure repeated through five passages. Lesions developed in every guinea pig in all five passages and in all three experimental groups. The lesions were in every respect characteristic of experimental fusospirochetal infections in guinea pigs. They included localized abscesses and spreading infections with abundant typical exudate and extensive necrosis. Comparable lesions were found in the control series.

75. Study of the Relationship of DMF to Certain Aspects of Personality as Measured by the Minnesota Multiphasic Personality Inventory. *J. H. Manhold and R. S. Melton, School of Aviation Medicine, Pensacola, Fla.* Psychosomatic considerations have been applied to many disease syndromes in the medical field. In 1949 and 1950 Manhold and Manhold reported similar preliminary results in dentistry. In 1953 Manhold expanded the previous work using more subjects and additional personality measures. Results showed the necessity of investigating deeper strata of personality. This study makes such an attempt. DMF scores were related both to individual MMPI scale measurements and to a clinical evaluation of the total test pattern. The MMPI and DMF scores were obtained on six classes of Naval Aviation Cadets. DMF scores were computed from visual and roentgenographic surveys. MMPI scores were obtained in the accepted manner and clinical profiles prepared. Correlations between personality scores and DMF were calculated for the 259 subjects. Resultant MMPI-DMF correlations were not significant (5 per cent level of confidence adopted). Significant differences in the number of extreme MMPI deviants were found, however, on a clinical analysis of the test profiles of the top and bottom 16 per cent of DMF scores (+ and − one standard deviation). Results on these eighty-two cases were: high DMF, thirty-six normal and five deviant cases; low DMF, twenty-four normal and seventeen deviant. A chi square statistical test proved significant at the 1 per cent level of confidence. Further testing of the relationship between personality variables and DMF is in progress.

76. Inhibition of Acid Production of Salivary Sediments by Various Ethers, Esters, Phenols and Alcohols. *R. S. Manly and G. L. Hargreaves, Tufts College Dental School, Boston, Mass.* A survey has been made of a large number of chemicals in order to determine whether any would possess the ability to reduce the acid formation rate of salivary sediment. The substances were tested in water or 10 per cent propylene glycol at concentrations ranging from 0.1 saturated to 1 per cent by a method of Manly and Hargreaves. Of seventy-one compounds having ether or ester linkage only, there was only one which depressed acid production by more than 60 per

cent. Of the 229 tests on alcohols, sometimes containing ether or ester, there were forty-two substances permitting recovery of acid production by an extent of less than 40 per cent of the original. All of these substances required the presence of propylene glycol for their activity to be disclosed. This solvent had a synergistic effect on four compounds tested at 1 per cent and had a combined solvent and synergistic action on ten others. Of the phenols tried at the maximum concentration, none of the derivatives were superior to phenol itself. The mono- and diethylene glycol ethers of the alcohols possessed either the same or less activity than the original alcohol. There were a few 1,2 dihydroxy compounds showing activity, and among these were monopropionin and monobutyrin. Several of the compounds showing activity bore considerable resemblance to the pyranose structure for glucose.

77. Periodontal Disease: Prevalence and Incidence. *C. D. Marshall-Day, Russell G. Stephens and Lawrence F. Quigley, Jr., Tufts College Dental School, Boston, Mass.* As part of a more comprehensive study of the epidemiologic characteristics of periodontal disease, the present report deals with the prevalence and incidence of the disease in a representative sample of the population in Boston, in the age range 13 to 65 years. Clinical and roentgenographic examinations of 1,300 subjects revealed a much greater prevalence of gingivitis and alveolar bone loss than has been generally reported. The incidence of gingivitis slowly increased from the high level of 80 per cent at age 13, to 95 per cent at age 65 years, but decreasing to 62 per cent at age 19 to 22 years. In ten of the thirteen age groups examined, the women showed a lower prevalence than the men, the difference in the other three age groups being relatively insignificant. The greatest difference was in the 19 to 22-year age group where the figures were 48 per cent and 76 per cent for the women and men, respectively. The prevalence of periodontal disease with roentgenographic evidence of bone loss involving one or more teeth ranged from 4 per cent at 13 to 15 years to 100 per cent at age 65 years. From age 35 years prevalence of bone loss was almost universal, ranging from 98 to 100 per cent. These figures include all degrees of bone loss from slight localized to severe generalized involvement. The pattern of chronic destructive periodontal disease showed a predominance of slight-localized bone loss up to the age of 35 years. From that age onward there was a constantly increasing incidence of moderate to severe generalized bone loss. Bone loss without demonstrable gingivitis was rarely encountered. The number of cases showing the loss of one or more teeth increased to 100 per cent at age 40, the number of missing teeth ranging from 6 per cent at age 19 to 22 years to 60 per cent at age 60 years, with a marked increase in tooth mortality at age 40 years. This aspect of our study indicates the need for greater emphasis on early diagnosis and treatment of periodontal disease.

78. Relationship of Glucosamine Levels to Lactobacillus Population in Human Saliva. *Morris M. Matt, School of Dentistry, Western Reserve University, Cleveland, Ohio.* It has been hypothesized at this laboratory that the mucin of saliva (a carbohydrate-protein complex) may be depolymerized and liberate a constant source of proteins and carbohydrates. These products may serve as a substrate for the oral flora (e.g., lactic acid formers). Rogers has demonstrated in 5 samples of raw saliva the utilization of glucosamine. This has been confirmed upon 7 different samples of stimulated saliva. In the presence of bactericides, phenol and fluorine, or by heating to 100° C. for ten minutes, the utilization of glucosamine was completely inhibited. It had been demonstrated that precipitated salivary mucin may serve as an efficient source of essential amino acids for the oral lactobacillus in the presence of a

source of proteolytic or amylolytic enzymes capable of hydrolyzing this glycoprotein. Previous experiments on the relationship of salivary mucopolysaccharide (estimated by the periodic acid Schiff reaction) to the total caries experience of the individual, indicated that salivary mucin may be a source of nourishment for oral bacteria. The glucosamine content was determined by the modified Elson-Morgan technic for 22 human salivas and, concurrently, salivary lactobacillus counts were performed on all the salivas. They varied from 12 to 72 mg. glucosamine hydrochloride per 100 ml. of saliva (mean value 34 mg.). The lactobacillus counts varied from 0 to 184,000 per milliliter of saliva. A scatter diagram indicates that there may be a correlation between lactobacillus counts and glucosamine levels in human saliva; however, more data are necessary to determine if a relationship does exist.

79. The Rat Molar Method for the Study of Pulpal Response to Local Injury. *Charles G. Maurice and Isaac Schour. Departments of Dental Therapeutics and Histology, University of Illinois, College of Dentistry, Chicago, Ill.* An experimental method of preparing and filling dentinal cavities in the maxillary right first molars of the albino rat was developed and standardized. The cavities were prepared on the mesial surface immediately above the free marginal gingiva. Each cavity was circular in outline with a diameter of 0.5 mm. and a depth of 0.25 mm. A special operating table for opening the jaws and for positioning and immobilizing the head of the anesthetized rat was constructed. A strong spotlight for focusing on the site of operation and a dissecting microscope for adequate magnification were used. The enamel of the tooth was first penetrated with the tip of a No. 1 Kerr Dental Manufacturing Company diamond stone mounted in the handpiece of the dental engine. The cavity was then completed by rotating a bibeveled steel drill by hand. This was accomplished by rotating the drill by means of the thumb, index finger, and third finger of the operator's right hand. This drill was made by grinding off the cutting blades from the head of a No. 33½ inverted cone dental bur on a flat India stone and in their place grinding 4 planes, 2 of which were converging. Histologic sections, cut in a plane passing through the bifurcation between the anterior and mesiolingual roots through the distobuccal root showed the cavity outline and the longitudinal course of the tubules from the cavity to the subjacent pulp tissues. This method is useful for biologic screening of the effect of different filling materials and drugs upon the pulp.

80. Reactions of Powdered Enamel and Dentin. *Harold G. McCann and Frederick A. Bullock, National Institute of Dental Research, Bethesda 14, Md.* Samples of powdered, fat-free, unashed samples of enamel and dentin were treated with sodium fluoride solutions over the range 2 ppm to 2 per cent F. Analysis of the solid and liquid phases enabled calculations to be made indicating the types of reactions which had taken place. Fluoride ion reacts with dentin or ions eluted or dissolved from dentin in several different ways: namely, (a) possible exchange with CO_2 on the crystal surfaces; (b) direct adsorption; (c) precipitation as MgF_2 of the magnesium released from crystal surfaces; (d) double decomposition at high fluoride levels to form CaF_2; and (e) formation of fluorapatite by exchange with hydroxyl ion. Enamel reacts similarly but to a much more limited extent. The approximate amount of fluorine contributed by each of these reactions has been estimated. It is shown that the principal reaction up to 100 ppm F is the formation of fluorapatite in enamel, dentin, and synthetic hydroxyapatite.

81. A Clinical Study of Hemodynamic Pressor Responses to Certain Epinephrine-Containing Local Anesthetic Solutions. *Frank M. McCarthy, School of Dentistry, Georgetown University, Washington, D. C.* Two groups

of 20 normal adult men were studied. Palatine injections were administered to one group and maxillary mucobuccal fold injections were administered to the second group. Each of 6 procedures was performed on each subject: the injection of 4 per cent procaine hydrochloride plain, and with epinephrine hydrochloride 1:50,000; the injection of 2 per cent lidocaine hydrochloride plain, and with epinephrine hydrochloride 1:100,000; the injection of isotonic saline; and a pseudo injection during which the usual routine was followed except for the lack of needle penetration. Blood pressure was determined by the cuff method immediately before, and at one-minute intervals for ten minutes following each injection. In only a negligible number of cases was a pressure rise noted. Little difference was noted when the results of each procedure were compared, thus demonstrating that the epinephrine had no systemic effect. The possibility that the palatine injection of a local anesthetic solution containing epinephrine increases the incidence of systemic effects, as suggested by previous animal experimentation, was not substantiated. The psychic factor inherent in the use of local anesthesia was demonstrated, and it was suggested that patient apprehension should be the primary concern when considering hemodynamic pressor responses associated with the use of epinephrine-containing local anesthetic solutions.

82. The Effect of Aspirin on Edema and Edema Fluid. *Ilmar Merits, S. C. Harris, and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* Insofar as aspirin has little or no effect on the pain threshold as determined by induced pain, it was postulated that the effect of aspirin on certain types of pain is to reduce edema, thereby reducing the pressure and hence reduce pain. This is especially true in toothache, arthritic pain, and possibly headache. Experimentally induced edema was produced by the injection of $AgNO_3$ into the ankle joint of the rat. The local interstitial fluid of the leg increased in volume by 5 ml. This was accompanied by a tenfold increase in protein concentration. When aspirin was given at the same time as the $AgNO_3$ the edema was partially aborted. If aspirin was given after the edema was produced there was a shift of proteins from the interstitial fluid and the edema was reduced. The results are indicative that aspirin acts on the cause of pain rather than on the pain threshold. More experiments concerning the source of interstitial protein and perhaps other constituents are necessary before the mechanism of action can be explained.

83. Replantation of Teeth in the Hamster. *Hugh I. Myers, Leo Nassimbene, Jim Alley, and John Gehrig. (Technical assistance by Viola Flanagan.) University of Kansas City, School of Dentistry, Kansas City, Mo.* Eight littermate hamsters, 36 days old, were secured, and replantation operative procedures performed within two weeks thereafter. Using Nembutal anesthesia, bilateral mandibular second or third molar extractions were made, and the teeth immediately replaced in their original sockets. At this age, these teeth are fully erupted and root growth is essentially complete. One animal died in less than twenty-four hours. The other seven were sacrificed postoperatively as follows: Second molar replants—one animal, five days; one animal, ten days; one animal, twenty-one days; and one animal, thirty days. Animals with third molar replants—one animal, five days; one animal, ten days; and one animal, nineteen days. The mandibles were removed, fixed in formalin, electrolytically decalcified, paraffin embedded, sectioned, every tenth section mounted and stained with hematoxylin and eosin. Microscopically, the replanted teeth in the animal which died in less than twenty-four hours showed an avascular pulp and a prominent space between the torn periodontal membrane and the cementum. One of the remaining fourteen teeth was lost during decalcification. The remaining thirteen teeth all showed excellent periodontal membrane regenera-

tion. In the five-day animals, some of the periodontal membrane next to the cementum showed relatively immature collagenous tissue, but in the later stages, heavy well-oriented fibers were universal. The pulps in the same thirteen teeth showed viable tissue with odontoblasts in ten. The other three had completely necrosed pulps. In some instances, the pulps in the five- and ten-day animals were viable and well vascularized in the root canal, but not in the coronal portion.

84. Caries Resistance in Hamsters. *David F. Mitchell, School of Dentistry, University of Minnesota, Minneapolis, Minn.* The results of a study of sixty-seven hamster offspring subjected to the prenatal influence of sugar versus fluoride were questionable due to low caries scores in each group after 150 days of a "caries-producing" diet. A study involving 120 animals, designed to delineate the effects of ingested urea on caries incidence, was invalidated due to insufficient caries in the experimental and control groups. A repeat study of eighty animals from another source especially bred and fed a high sugar diet during the period of 8 to 21 days of age (after Volker: *J. D. Res.* **31**: 478, 1952, animals obtained from same source) before and during eruption of molar teeth, has now been maintained 250 days without developing appreciable amounts of caries in either group detectable in vivo. Thirty-two of these animals were sacrificed at 250 days and scores were exceedingly low. The remainder will be continued indefinitely to see if susceptibility to caries increases. These studies point up the need to re-establish a high degree of caries susceptibility in our hamsters. The problem is not unique to our colony alone. Animals from four sources more recently obtained and similarly maintained have shown minimal caries susceptibility as observed in vivo, except for one group, which is uniformly highly susceptible after a period of only eighty days. The latter animals are being in-bred.

85. The Effect of Various Inorganic Salts on Experimental Caries in the Rat. *Joseph C. Muhler and Harry G. Day, Department of Chemistry and School of Dentistry, Indiana University, Bloomington, Ind.* Nine different inorganic fluorides were fed to rats in the drinking water, under various conditions of solution pH and concentration, to determine the anticariogenic ability of each. All of the aimals received the same cariogenic stock corn diet and were compared to appropriate controls not receiving added fluorides. The femurs from each group were analyzed in order to determine the storage of fluorine in the skeleton. The dental caries data indicated considerable variability existed between different fluorides fed in the drinking water and at the same fluorine concentration. In all the experiments, stannous fluoride was the most effective. Hydrofluoric acid and sodium fluoride were about equal in their effectiveness, but about one-half as effective as stannous fluoride. Sodium monofluorophosphate was ineffective, while potassium fluoride and sodium fluoride were about equally effective. Stannic fluoride was more effective than sodium hexafluorophosphate, but not nearly as effective as stannous fluoride. No relationship between the caries-reducing ability of an inorganic fluoride and its fluorine storage in the skeleton was possible from these data.

86. Restriction of Dietary Calcium Intake During the Period of Molar Calcification in the Suckling Rat. *Paul L. Munson, Svein U. Toverud, and Alexander D. Kenny, Biological Research Laboratories, Harvard School of Dental Medicine, Boston, Mass.* Calcification of the molar teeth of the rat is essentially complete at the end of the normal suckling period. Thus, in order to reduce calcium available for molar tooth formation as a preliminary to a test for effect on caries susceptibility it appeared necessary to decrease the calcium content of the mother's milk. Two methods were employed in an

attempt to accomplish this result: restriction of the mother's dietary calcium intake and parathyroidectomy. Milk from six rats on a normal stock diet analyzed 3.19 ± 0.14, 3.12 ± 0.15, and 3.10 ± 0.07 mg. calcium per gram on the thirteenth, fifteenth, and nineteenth days of lactation, respectively. The corresponding values for eleven rats placed on a low-calcium diet on the eleventh day of lactation were 2.21 ± 0.09, 2.15 ± 0.11, and 2.04 ± 0.08, a decrease of approximately 30 per cent. A similar decrease in milk calcium concentration was observed in a second, confirmatory experiment. Representative young are under observation on the cariogenic diet of Shaw. Others were killed and femurs and jaws were preserved for detailed examination and analysis. Grossly, the femurs from the low-calcium group were fragile and appeared to be drastically acalcified. A small number of rats on the normal diet were parathyroidectomized on the eleventh day of lactation. Two days later there was a greatly diminished milk flow and a markedly reduced mean serum calcium, but no reduction in milk calcium concentration. Hence, parathyroidectomy does not answer the requirement of the present study. However, further investigation of the role of the parathyroids in lactation is warranted.

87. Phosphorus Exchange Rate in Enamel as Influenced by Chewing. *H. H. Neumann and N. A. DiSalvo, College of Physicians and Surgeons, Columbia University, New York, N. Y.* The phosphorus turnover in enamel was compared on the chewing and nonchewing half dentitions of rhesus monkeys. The monkeys were forced to chew on one side only by varying surgical means, as unilateral masticatory muscle resection, nerve resection, or quadrant of teeth resection. The rate of the phosphorus turnover was followed by the use of P^{32} and a small pencil shaped end-window Geiger-Müller counter. The measurements were made topically at frequent intervals over a period of fourteen months. Normally no difference was found in the phosphorus exchange rate in left and right molars in monkeys. With unilateral chewing certain trends were apparent: the phosphorus exchange rate in the less functioning molars was increased and the difference reached a maximum of over twice the level of the chewing molars. This change was not permanent and after several months the rates became again equal, with a subsequent reversal of the first found trend: the turnover rate in the nonfunctioning molars fell steadily, well below the level of the functioning contralateral teeth. It appears from the findings that the exchange rate of phosphorus ions in the tooth is affected by biologic variables and that function manifests itself as a factor influencing the ionic balance and turnover rate.

88. Reducing Property of Saliva Towards Some Oxidation-Reduction Indicators. *Gordon Nikiforuk, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* A wide variety of biologic processes are intimately linked with oxidation-reduction potentials. One method for quantitatively studying these processes is the use of oxidation-reduction dye indicators. Saliva reduces redox indicators whose E° (potentials of half reduced dyes at pH 7.0) is more positive than approximately –0.20 v. The rate at which equimolar solutions of some dyes is reduced is indicated by the following series: Toluylene blue faster than (>) methylene blue > 2, 3, 5 triphenyl tetrazoium chloride > cresyl violet > safranin (only slightly reduced). The fraction of the saliva that exhibits reducing properties is associated with the insoluble sediment. A significant correlation between the amount of sediment and the reducing coefficient of saliva has been shown. The reducing capacity of saliva is decreased 50 per cent by Furacin (4.2×10^{-5} M); iodoacetate (1.3×10^{-4} M) and cyanide (7.3×10^{-6} M). Sodium fluoride and penicillin had no

effect. No demonstrable effect was observed by adding glutamate, succinate, or formate. The reducing coefficient of saliva is not sharply influenced by pH. A broad pH optimum is between pH 7 and 8.

89. A Quantitative Technique for the Study of Precipitate Formation in Bacterial Cultures. *William M. O'Leary, University of Pittsburgh, School of Dentistry, Pittsburgh, Pa.* Precipitate formation by microorganisms in calcium-rich media has been reported as indicating that bacteria play an active role in calculus formation, although most reports offer no quantitative data. A quantitative method has been developed to assess the importance of microorganisms in precipitate formation. A suitable medium for such studies should support growth of most oral organisms and should form a minimum of nonspecific precipitate. A beef heart dextrose infusion broth was devised which approached these ideals. In these preliminary studies, the cultures, after suitable incubation, were filtered through ashless paper and ignited. The inorganic residue would represent that from the microorganisms, that from spontaneous precipitation, and that presumably due to the precipitating action of the microorganisms. The amount of the latter could be obtained by subtracting inorganic material from suitable control tubes. Pure cultures of *Actinomyces bovis* and mixed cultures of six nonfilamentous aerobic oral bacteria have been used. In each case the amount of precipitate recovered from inoculated media containing added calcium chloride was greater than that recovered either from sterile controls or from inoculated media containing no added calcium chloride. Precipitates from *Actinomyces bovis* cultures exceeded those from mixed cultures. Actinomyces culture pH's generally were slightly more acid than those of mixed cultures, although they rarely went below pH 6.6. For quantitative calcium and phosphorus determinations this technique was refined by extracting precipitates with 2M acetic acid. The extracts were evaporated, ashed, and weighed. Results were quantitatively similar to those of earlier experiments, except that higher residue weights were found in cultures of mixed organisms and actinomyces culture pH's were low (6.0-6.3) compared with mixed cultures (7.4 to 8.1). The residues were dissolved in trichloroacetic acid and their calcium and phosphorus contents were determined. Residue contents of these elements were proportional to their respective residue weights. Results obtained by such methods show a desirable degree of reproducibility and sensitivity and suggest that these methods may find application in the study of the role of bacteria in calculus formation.

90. A Study of the Nerve Supply to the Upper Anterior Teeth. *Norman H. Olsen, University of Kansas City Dental School, Kansas City, Mo.* There is a variance in the description given by anatomists of the innervation to the upper anterior teeth. It is apparent that the nerve supply to the maxilla is not as constant as that found for the mandible. The controversial point in giving the nerve innervation to the upper anterior dentition has been whether the anterior superior alveolar nerve is the sole source of innervation, whether the nerve supply is received partly from the nasopalatine nerve, or whether there is a combination of the two. The objective of this study was to determine the actual source of innervation to the upper anterior teeth. Variations of the nerves in the area were noted together with other pertinent data observed during the source of the study. Cadaver material was employed in this study, and the twenty-six specimens were selected on the basis of the number of upper anterior teeth present and the presence of the nasal septum. The electrolytic form of decalcification was used because of its rapidity and the size of material to be decalcified. Gross dissections were performed on the specimens upon completion of the decalcification. Some of the observations made

were: 1. Degree of variance as to the origin of the anterior superior alveolar nerve from the infraorbital nerve. 2. The variation in the complexity or sparseness of the nerves. 3. Absence of the middle superior alveolar nerve in 53 per cent of the cases. 4. A nerve branch arising from the nasopalatine nerve in incisive canal in 26 per cent of cases. 5. Variation in the number of nerve branches arising from the infraorbital nerve, constituting the anterior superior alveolar nerves. 6. Presence of a plexus between the anterior and posterior superior alveolar nerves in absence of the middle superior alveolar nerve. 7. Nerves of tactile sensibility between the adjacent teeth are larger in diameter than those innervating the teeth.

91. Electron Micrographic Observations of Sectioned and Unsectioned Oral Bacteria. *Frank J. Orland and Wayne Ferris, Zoller Memorial Dental Clinic and the Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.* Although the trend of interest in this research center has largely shifted from "what bacteria look like to what bacteria do," it is still important to attempt to gain a greater insight into bacterial morphology. With the two new tools now adaptable to biological materials, the electron microscope and the ultrathin sectioning microtome, it has been feasible to view certain aspects of oral microorganisms never before possible. Some of the more common microbial forms found in the mouth of man and certain animals were studied and photographed under the electron microscope. Some cultures of these oral bacteria and yeasts were fixed by osmium tetroxide or neutral formalin, chromium shadowed, and then attached to the grid screen of the microscope. Others were fixed by OsO_4, dehydrated through a graded alcohol series, embedded in butyl methacrylate, and cut into ultrathin sections on a special glass knife microtome. Both types of preparations were viewed under the various high magnifications of the electron microscope. Observations made indicate a variety of external and internal structures of oral bacteria and yeasts.

92. The Oral Administration of Lactobacillus Acidophilus Into the Syrian Hamster. I. Oral Lactobacilli Population in Relation to Dental Caries. *Hubert B. Palmer, USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Tex. Norman C. Laffer, and John E. Faber, Jr., Bacteriology Department, University of Maryland, College Park, Md.* Twenty-four Syrian hamsters, 23 days old, were segregated according to sex and littermates into four dietary groups: regular (R), regular and lactobacilli (RL), cariogenic (C), and cariogenic and lactobacilli (CL). All animals received Purina Laboratory Chow and tap water ad libitum. Those in the C and CL groups received, in addition to the basal diet, 5 grams of chemically pure dextrose daily. Animals of RL and CL groups received daily 0.5 to 1.0 ml. (volume increasing with age) saline suspension of *L. acidophilus* in concentration of 9×10^8 cells per milliliter. Salivary lactobacilli counts, using Rogosa's medium incubated anaerobically for seventy-two hours at 37° C., were made biweekly. Saliva samples were procured between eighteen and twenty hours following oral inoculation of lactobacilli. Animals at 29 weeks of age were anesthetized and sacrificed. For each animal the total volume of all carious areas seen at magnification 21× were scored and expressed as the caries index. Lactobacilli counts of C and CL groups were generally higher (in some instances significantly so) than the R and RL groups during the various study intervals of the experiment. Counts of RL group were significantly higher than of R group (CR 2.40) early in the study and significantly lower (CR 2.22) later. There was no statistical difference in counts between C and CL groups (CR 0.18). The mean caries indices for the four groups were R—2.26, RL—1.50, C—7.16, and CL—4.60.

93. An Investigation of the Relation of Nutrition to Tooth Morphology. *K. J. Paynter, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada, and R. M. Grainger, Division of Medical Statistics, Ontario Department of Health.* Because about 75 per cent of all decay in the permanent teeth of children occurs in the pits and fissures, it was important to determine whether the origin of these hypersusceptible areas was purely genetic, or the result of some metabolic disturbance at the time of tooth formation. The effects of Vitamin A and Vitamin D deficiency and of additional fluoride in drinking water were studied in relation to rat molar size and to the characteristics of the pits and fissures. Female Wistar rats were placed into one of four groups. Group I (control) was fed a complete synthetic diet; Group II a diet completely deficient in Vitamin A for a period of six weeks to deplete liver reserves and then a diet containing a suboptimal level of the vitamin; Group III received the complete diet to which had been added sufficient Ca to raise the Ca/P ratio to 4:1 to simulate a vitamin D deficiency; and Group IV received a diet with both the A and D deficiency. One-half the animals in each group received only distilled water to drink, the other half, distilled water to which had been added NaF at a concentration of 10 ppm. Litters resulting from mating with normal males of the same strain were raised to the age of 21 to 25 days. After sacrifice, heads were placed in 10 per cent formalin. The upper right first molar of each specimen was dissected and photographed under the same conditions. Preliminary analysis of measurements of five different dimensions yielded the following results: If in the diet of female rats the Vitamin A intake was reduced to a suboptimal level, or if Ca was added to raise the Ca/P ratio to 4:1, or if NaF was added to the drinking water, molar teeth in young born to these animals were significantly smaller than those of controls. There was a strong indication that the distal fissure in the teeth of young whose mothers were given NaF was shallower than normal. Dimension changes were in the order of about 5 per cent.

94. Cytochrome Oxidase and Succinoxidase Activity in Oral Tissues. *Philip Person and George W. Burnett, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* Preliminary to the study of the metabolism of normal and diseased oral tissues, it was necessary to determine whether existing techniques were suited to their investigation. Cytochrome oxidase and succinoxidase activity of tissue homogenates of tongue, parotid and submaxillary gland, liver, heart, and kidney of adult white rats was determined at 37° C. by conventional manometric techniques. Cytochrome oxidase activity was determined with hydroquinone as substrate, air as gas phase, KH_2PO_4-Na_2HPO_4 buffer system, cytochrome c in excess, and semicarbizide-HCl for quinone fixation. Succinoxidase activity was determined with sodium succinate as substrate, together with added Ca^{++} and Al^{+++}, air as gas phase, and cytochrome c in excess. Except for minor variations, the cytochrome oxidase activity of the various tissues was optimal from 0.15 to 0.25 M buffer concentrations and from pH 6.8 to 7.1. Succinoxidase was most active in circa 0.2 M buffer concentration and from pH 7.2 to 7.8, but the range of optimal activity with regard to buffer concentration varied more in the different tissues than it did for cytochrome oxidase. Succinoxidase activity when measured manometrically and spectrophotometrically was absent from homogenates of guinea pig alveolar bone previously freed from periosteum, marrow, and periodontal membrane. In similar homogenates cytochrome oxidase activity was absent when measured spectrophotometrically, while manometrically the low oxygen consumption rendered conclusions as to the presence of the enzyme equivocal. The "Nadi" reaction for cytochrome

oxidase, when applied to bone slices, also gave equivocal results. These findings indicate that modification of existing techniques for study of intermediary metabolism may be necessary when adapting them to oral tissues.

95. Dentinal Attack in the Artificial Mouth. *Ward Pigman, William Hawkins, Joe Watson, Ralph Powell and Carolyn Gaston, University of Alabama, Dental School, Birmingham, Ala.* When extracted teeth are innoculated with saliva and exposed to a continually replaced bacteriologic medium, the nature of the attack on the teeth is greatly influenced by the concentration of the glucose in the medium. For glucose concentrations of 0.5 per cent, decalcification and loss of enamel in noncleansed areas occurs rapidly. For glucose concentrations of 0.0 and 0.125 per cent, no action on sound tooth structures takes place, but previously decalcified dentin is destroyed in one or two weeks at 35° C. These results agree generally with those reported earlier by Burnett and Scherp for blocks of enamel and dentin. In a restricted concentration range around 0.3 per cent glucose, decalcification and dentinal matrix destruction occur simultaneously. At all glucose concentrations, streptococci are present on the tooth surfaces, but lactobacilli require the presence of a minimum of about 0.25 per cent glucose. The basic medium used in this work was composed of 0.5 per cent tryptone, 0.2 per cent yeast extract, and 0.0125 per cent $CaHPO_4 \cdot 2H_2O$ at pH 6.4.

96. A Technic for the Localization of Brain Centers Concerned With Dental Neurophysiological Phenomena. *Charles S. Powell and Joseph A. Gibilisco, University of Alabama, School of Dentistry, Birmingham, Alabama.* One of the major difficulties encountered by investigators of dental neurological problems is the lack of a reliable method for localizing those areas of the brain associated with the nervous activity of the oral cavity. Formerly extensive surgical procedures have been employed to gain access to desired areas. These methods, however, do not lend themselves to clinical observations. We are currently engaged in similar researches using a stereotaxic instrument. This device offers a means of inserting electrodes into the specific areas of the brain without excision of any brain tissue. It further acts as an animal head holder and stabilizes the electrodes so that once inserted they cannot be moved. By means of determining a series of coordinates the instrument can be readily adapted from one animal to another, or used for repeated experiments on the same animal. To date it has been possible to calculate a series of coordinates which allow the insertion of electrodes into the Gasserian ganglion. This has permitted the oral tissues to be electronically stimulated and the nervous impulses passing through the ganglion have been picked up by the electrodes and recorded. The animals have recovered without significant loss of function. To verify the location of the electrode puncture some of the ganglia have been dissected and examined histologically.

97. Cephalometric Roentgenography of Infants. *Samuel Pruzansky and Edward F. Lis, National Institute of Dental Research and the Cleft Palate Center, Chicago Professional Colleges of the University of Illinois, Chicago, Ill.* The rate of growth of the head during the first year of life is far greater than that observed during later periods of life. Despite the development of roentgenographic cephalometry, comparatively little information has emerged concerning the patterns of normal and abnormal growth of the cranium and face during this early period of life. Such information, if available, might assist in the management of various congenital anomalies, traumatic injuries, and infections that involve the structures of the head. Two principal reasons exist for the paucity of roentgenographic cephalometric studies of the newborn infant: The lack of familiarity with simple and safe technics for the

sedation of infants and the inability to adapt existing cephalometric equipment for accurate roentgenographic cephalometry of the infant. The sedative of choice is a barbiturate in aqueous suspension, given as a retention enema. Presedation and postsedation instructions are given to the parents. Individual variations have been observed in the induction and emergence from sedation. In general, barbiturate sedation is a relatively innocuous procedure. Brain damage; respiratory obstructions due to infections, micrognathia, glossoptosis, laryngotracheal-bronchial anomalies; parenchymatous lung involvement, and kidney and liver immaturity or dysfunction are either relative or absolute contraindications to barbiturate sedation. The cephalometer consists of a table for the supine positioning of the infant and two x-ray tubes mounted in fixed relation to a head-hold device. The x-ray tube for the lateral view is situated to provide for a five-foot distance from the focal spot to the mid-sagittal plane. A correctional scale is mounted at the mid-sagittal plane and is included in each lateral film. For the posteroanterior view, the x-ray tube is positioned at a distance of three feet from the transmeatal axis.

98. Effect of Reversals in Diagnosis on the Interpretation of Clinical Dental Caries Tests. *Arthur W. Radike, United States Air Force, Ohio State University, Columbus, Ohio, and Joseph C. Muhler, Indiana University, Bloomington, Ind.* In a clinical dental caries test it is important to maintain minimum variability in the examination procedure. This is most difficult in diagnosing those conditions which are neither clearly carious nor clearly noncarious. Some of these conditions are equally likely to be judged carious or noncarious and are referred to as borderline conditions. Random judgment of borderline conditions results in tooth areas being diagnosed carious at the initial examination and noncarious at the final examination. These inconsistencies are easily detected and are called reversals in diagnosis. It can be demonstrated that one-fourth of the borderline conditions which remain unchanged during the test period result in reversals in diagnosis. The number of borderline conditions which are actually carious at the initial examination and which subsequently show reversals in diagnosis represent four times that number of nonprogressive or arrested incipient lesions. Study of clinical caries test data from three separate sources resulted in the following conclusions: 1. reversals in diagnosis must be included in test data in order to obtain the best estimate of the over-all effect of an anticaries treatment. When reversals are altered or excluded from the data a false effect is introduced. 2. Borderline conditions and reversals in diagnosis occur with appreciable frequency and may significantly affect the result of a test. 3. Reversal data may give some indication of the relative degree of incipient caries arrest among groups.

99. The Gingival Sulcus and the Periodontal Pocket Immediately Following Scaling of Teeth. *Sigurd Ramfjord and Gordon Kiester, University of Michigan, School of Dentistry, Ann Arbor, Mich.* A wide variety of opinions have been expressed on the nature and response of the epithelial attachment to therapeutic procedures. This report deals with the histologic findings in nine specimens obtained immediately following scaling of teeth for removal of plaques and subgingival calculus. The specimens were obtained from patients who needed partial resection of the alveolar process in association with insertion of dentures. It was found that thoroughly executed routine scaling of teeth with fine instruments severed the epithelial attachment and that this cut commonly extended into the adjacent connective tissue attachment. Aggregations of surface granulation tissue and epithelial cells were loosened and sometimes removed from their normal site. The scaling also tended to split deep rete pegs in the wall of the pocket. Special stains for bacteria revealed the presence of bacteria on the surface of the specimens, and within areas of debris and extravasated blood. No invasion of bacteria into living tissue was found.

It was concluded that subgingival curettage or gingivectomy, if performed following thorough scaling, should be extended only to the bottom of the clinical pocket at that time, because the epithelial attachment has already been severed by the previous scaling. Scaling should be done with special care in areas of severe gingival inflammation to avoid deepening of the gingival crevice or the periodontal pocket.

100. A Screening Test for Mucin Adsorbing Enzyme Inhibitors. *G. W. Rapp, L. Blanchet, and B. Dusza, Loyola University School of Dentistry, Chicago, Ill.* Since mucin is a considerable component of the dental plaque, it was felt that an enzyme inhibitor which adsorbs on mucin from saliva might also adsorb on the dental plaque. A technic was devised which consists essentially of adding a solution of the inhibitor under test to freshly gathered human saliva, centrifuging sharply, washing the precipitate with suitable solutions, and adding substrate-containing solutions to the precipitate. Changes in the substrate are then measured after suitable incubation periods. Differences in native and altered mucin as regards their adsorbing capacity were found. Typical examples of effective and ineffective mucin-adsorbing inhibitors against glycolytic and proteolytic enzymes were given.

101. Correlation of the Mandibular Condyle Osteoclast Count With Age in the Guinea Pig. *Wayne L. Reeve and Hugh I. Myers (Technical assistance by Viola Flanagan), University of Kansas City, Dental School, Kansas City, Mo.* Normal healthy male guinea pigs ranging in age from 2 to 58 weeks were secured. The mandibular rami were removed, fixed, decalcified electrolytically, paraffin embedded, and serially sectioned, and stained with hematoxylin and eosin. Sagittal, frontal, or cross sections were made of the condylar region. The number of osteoclasts seen on every tenth section was tabulated. Sagittal, frontal, and cross sections all showed a constantly decreasing number of osteoclasts per slide with increasing age from 6 weeks through 58 weeks. The sections taken from animals 2 to 6 weeks of age show considerable more variability.

102. Preliminary Histological Observations of Oral Tissue Subjected to the Action of Collagenase. *Genevieve Roth and Hugh I. Myers, University of Kansas City, School of Dentistry, Kansas City, Mo.* Ten adult hamsters were given various concentrations of collagenase as subcutaneous injections into the hard palate and topical applications on the mandibular gingiva and floor of the mouth. The animals were sacrificed twenty-four hours after treatment, the tissues fixed in formalin, paraffin sectioned, and stained with hematoxylin and eosin. The entire palate of one animal was decalcified and serially sectioned, the other animals had the soft tissues stripped from the underlying bone for study without being subjected to the decalcifying process. The gross picture of the intact tissues after twenty-four hours showed no discernible deviation from the normal. However, when the fresh palatal tissue was incised for removal, a pronounced tendency for it to be separated from the underlying bone was noted. Microscopically the tissue that received subcutaneous injection (that is, palate) showed fragmentation and destruction of the collagenous fibers, hemorrhage as a result of collagenous fiber destruction in blood vessel walls, and a chronic inflammatory cell infiltration. This reaction tended to be concentrated in the deeper connective tissue layers next to the bone. The lamina propria was relatively unaffected. No alterations were noted in the epithelium. Sections from topical applications showed no alterations. Generalized systemic toxic effects of the collagenase were noted in all animals.

103. Collection of Separate Submaxillary and Sublingual Salivas in Man. *Leon H. Schneyer, School of Dentistry and Medical College of Alabama, University of Alabama, Birmingham, Ala.* Systematic investigation of salivary

constituents and of the process of salivary secretion in man requires the collection of separate salivary fractions. Until the present time it has not been possible, with any degree of convenience, to collect the submaxillary and sublingual secretions separately, even though mixing of the secretions due to anatomic relationships is relatively rare. Pickerill (1919) devised an appliance (segregator) for the collection of mixed submaxillary-sublingual saliva. It is the purpose of this report to describe a modification of the Pickerill appliance which permits collection of separate submaxillary and sublingual fractions. The new method involves (1) making an alginate impression of the teeth and floor of the mouth, (2) pouring a stone model, (3) blocking out on the model of the area of the plica sublingualis and of the orifices of the submaxillary ducts with base-plate wax to a height of several millimeters, and (4) making a cold-cured acrylic appliance to cover these blocked-out areas. Small-gauge plastic tubing is led from each of the three wax-filled acrylic chambers. After the acrylic has polymerized, the wax is removed. A separate impression and appliance must be made for each subject. With the appliance in place in the mouth, secretion pressure causes the flow of the sublingual saliva from the two lateral chambers and of submaxillary saliva from the medial chamber. Quantitative analysis of the salivas for endogenous and externally added materials indicates that no leakage of any significance occurs from chamber to chamber.

104. Production of B-Glucuronidase by Streptococci Isolated From the Gingival Pocket. *Stig D. Schultz-Haudt and Henry W. Scherp, Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Viridans streptococci may be one source of the hyaluronidase present in the gingival pocket in nonspecific gingivitis and implicated in the accompanying depolymerization of connective tissue ground substances. B-glucuronidase, which completes this hydrolysis, was sought in viridans streptococci (mitis and salivarius) isolated at random from subgingival deposits. Resting cell suspensions of standard density were used with phenolphthalein glucuronide as substrate. At least 6 strains were tested from each of 15 subjects, 10 of whom had clinically manifest gingivitis. None of 30 strains (25 mitis, 5 salivarius) from the 5 negative subjects produced the enzyme, nor did any of 19 strains (all mitis) from 3 subjects having a degenerative type of gingivitis. Ten of 37 mitis strains and 2 of 14 salivarius strains from the remaining 7 gingivitis subjects produced the enzyme. From 1 to 5 strains were positive from each subject. Additional tests on pooled strains (at least 10 colonies of each type) from 15 more subjects gave positive results from all 7 having gingivitis, but from only 1 of the 8 clinically normal. The enzyme appears to be endocellular, for there is a prolonged lag in hydrolysis of the substrate unless the cells are treated with chloroform. The enzyme appears to be not adaptive, for none of the 25 negative strains tested became positive when cultivated in the presence of menthol-glucuronide, glucuronolactone, acetyl glucosamine, or hyaluronic acid. Positive strains, however, were twice as active when cultivated in the presence of hyaluronic acid (1 strain) or menthol-glucuronide (2 strains).

105. Electron Microscopy of Sectioned Carious Enamel. *David B. Scott and James J. Kennedy, National Institute of Dental Research, Bethesda, Md.* It has been demonstrated in earlier studies (Scott and Albright: *J. D. Res.* **32**: 682, 1953) that some specimens of carious enamel can be sectioned for electron microscopy without preliminary artificial demineralization. A marked persistence of organic matrix in such softened enamel has been noted. Results of further observations of similar preparations support the concept that the

earliest structural changes in caries, as detectable in the bacteria-free "altered enamel" zone, are a result of mineral loss. Alterations in the organic components appear to occur subsequently.

106. The Effect of Hypophysectomy on Experimental Dental Caries and the Salivary Glands of the Rat. *William G. Shafer and Joseph C. Muhler, Indiana University School of Dentistry, Indianapolis and Bloomington, Ind.* The effects of hypophysectomy have been studied in a group of 39 operated rats (19 males and 20 females) with 45 littermate animals (22 males and 23 females) serving as controls. All animals were maintained on a caries-producing diet from birth. The experimental animals were operated on at 44 days of age and all animals were sacrificed at 181 days of age. Throughout the experimental period the operated animals consumed noticeably less food than the controls and, at sacrifice, weighed as follows: hypophysectomized males, 113 grams; control males, 330 grams; hypophysectomized females, 100 grams; control females, 194 grams. The control animals averaged 7.6 carious lesions, while hypophysectomized animals averaged 9.3 lesions. Proteolytic activity of the submaxillary glands showed marked diminution in hypophysectomized animals; in terms of relative activity, this was found to be: hypophysectomized males, 1.3; control males, 13.8; hypophysectomized females, 1.7; control females, 15.7. Amylase activity of the submaxillary glands was reduced in operated animals, although the range within each group was so wide that the results are of questionable significance. In terms of milligrams of reduced sugar per cubic milliliter of gland extract, this amylase activity was found to be: hypophysectomized males, 3.22; control males, 6.21; hypophysectomized females, 1.76; control females, 12.5. Histologic examination of the salivary glands has shown very marked alterations in the structure of the submaxillary gland of hypophysectomized animals, bearing considerable resemblance to those occurring after castration with nearly complete loss of granule-bearing tubules.

107. Inhibition of Enzymes by Fluoride. *Quenton Smith, Leon Singer, and W. D. Armstrong, Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.* The quantitative aspects of in vitro inhibition of salivary and human prostatic acid phosphatases and of yeast enolase by fluoride have been investigated. The degree of inhibition is directly proportional to the fluoride concentration and is exhibited by fluoride concentrations as low as 10^{-4} molar. The kinetics of the inhibition show that the effect of fluoride on the enolase enzyme is a result of combination with the metal activator, while in the case of the acid phosphatases the effect of fluoride appears to be competitive. It would be feasible to base a fluoride analytic method upon the inhibition of prostatic acid phosphatase.

108. Characterization of a Proteolytic Enzyme in the Submaxillary Gland of the White Rat. *Leo M. Sreebny, Department of Applied Materia Medica and Therapeutics and Division of Oral Pathology, College of Dentistry, University of Illinois, Chicago, Ill.* Saline or buffer extracts of frozen-dried rat submaxillary glands demonstrated protease activity against casein and azocoll. At least two specific proteolytic enzymes were present: one with an optimum at pH 2.5, the other at pH 8.5-9.6. Similar extracts from rat spleen, liver, pancreas, testis, kidney, muscle, and parotid and sublingual glands did not liberate color from azocoll. This report deals with the alkaline protease of the submaxillary glands. Quantitative estimations of enzyme activity were made from (1) the optical density of azocoll-enzyme preparations at 522 mμ and (2) the tyrosine liberated from casein-enzyme preparations (Folin-Ciocalteu reagent). The enzyme was active toward azocoll, casein, gelatin, and benzoyl-l-arginine amide; inactive toward gastric mucin, chondroitin sulfate, and native

collagen. The pH optimum for azocoll was 8.5 to 9.6, with activity dropping rapidly on either side of the optimum. For casein, the optimum pH was 9.6; activity dropped off slowly from pH 7 to 5, then rapidly from pH 5 to 3. The alkaline protease is relatively thermostable; optimum temperature=50 to 55° C. It is not destroyed by heating at 75° C. for fourteen minutes, but is completely destroyed by similar treatment at 100° C. In the reaction with casein, at pH 9.6, the enzyme was activated by cysteine and ascorbic acid and inhibited by iodine; iodoacetic acid and $MgSO_4$ had no effect. Against azocoll, trypsin inhibitor and $MgSO_4$ had no effect. Dialysis of the enzyme extract resulted in a decrease in enzyme activity due to the loss of some inorganic component.

109. Some Histochemical Observations on Cornification. *Reidar F. Sognnaes, Harvard School of Dental Medicine, Boston, Mass.* Three types of material have been studied to date: (1) various epithelial derivatives ranging from hair to horn from various species, a study which is still in progress in collaboration with Barrnett and Pettengill; (2) the horny teeth of the lamprey, a joint study with Lustig; (3) the teeth of rat, monkey, and man. All the cornifying tissues show the presence of sulfhydryl groups in the malpighian layer attributed to the presence of cysteine. Hard and soft keratins could not necessarily be separated by a sharp transition from SH to SS groups. In the rhinocerus horn, in nails, claws, and feathers, the presence of SH groups can be demonstrated throughout, including the cornified layer. The horny "teeth" situated along the buccal mucosa and on the tongue of the lamprey presented a particularly ideal material for studies of histochemical reactions associated with the process of cornification. The first evidence of cornification was associated with the deposition of sulfhydryl-positive granules in the cytoplasm of the squamous epithelial cells adjacent to the horny groove. This process was associated with an intense nuclear alkaline phosphatase reaction but unaccompanied by deposition of minerals. As the horny caps matured, a positive reaction for disulfide bonds became evident, accompanied by brilliant birefringence when examined with polarized light. In the teeth of higher animals, the disulfide bond reaction in mature enamel is extremely weak, compared to other epithelial derivatives, presumably because of the very low cystine content of enamel. The sulfhydryl reaction in young enamel matrix is, however, more conspicuous than would be expected from the subsequent SS reaction. It is finally of interest that a SH reaction is present in the odontogenic cells and that this reaction to a lesser extent is also present in predentin, a structure which is not subject to cornification. This predentin reaction for Sh groups as well as part of the SH reaction in young enamel matrix must therefore be attributed to another function of SH groups, yet to be determined.

110. Purification of Enamel Protein Fractions. *Maurice V. Stack, Department of Dental Medicine, Guy's Hospital, London, England (now at Harvard School of Dental Medicine, Boston, Mass.).* Pooled enamel (99.8 per cent pure) was submitted to dialytic demineralization, and the protein fractions were separated by centrifugation (*J. D. Res.* **32**: 734, 1953). Treatment of the insoluble protein fraction with collagenase, pepsin, trypsin, or chymotrypsin resulted in the solubilization of four-fifths of this fraction. Preparations treated with boiling water for several hours were more satisfactorily purified from collagenous impurities, but the residual protein (four-fifths) still contained 3 per cent hydroxyproline (see Hess, Lee and Neidig, *J. D. Res.* **32**: 585, 1953). The amino acid composition was then evaluated (with 98 per cent recovery) by quantitative elution chromatography from a sulfonated polystyrene resin. The soluble protein fraction was purified by electrophoresis on filter paper which removed a similar collagenous impurity. It was concluded

that one-third of the 0.09 per cent collagenous material present in these enamel samples was associated with the insoluble fraction, and two-thirds with the soluble protein fraction.

111. The Development of Caries in Different Strains of Rats. *Robert M. Stephan and M. Rachel Harris, National Institute of Dental Research, Bethesda, Md.* It has been observed in a large series of experiments that a varying proportion of albino rats of the Sprague-Dawley and Holtzman strains from the National Institutes of Health stock colonies will develop caries when fed Diet 580. This is a fine-particle diet consisting of 66 per cent sucrose, 32 per cent dried skim milk powder, and 2 per cent dried liver substance. All types of carious lesions not involving fractures have been repeatedly produced with it, but within the same experiment some animals have developed caries whereas others have not. That some of this variation may be inherent within the stock colony has been indicated by experiments in which different strains of animals all raised on the same stock diet have been compared. Thirty-four females each of four different strains of rats from the National Institutes of Health stock colonies were marked for identification and distributed in thirty-four cages so that each cage contained four animals, one from each strain. They were fed Diet 580 for fifty-six days, then were sacrificed, the heads cleaned, and the occurrence of carious lesions observed under the binocular microscope. The following table summarizes the results:

STRAIN	NO. OF ANIMALS	NO. OF ANIMALS WITH CARIES	NO. OF CARIOUS TEETH	NO. OF OCCLUSAL SURFACE LESIONS	NO. OF PROXIMAL SURFACE LESIONS	NO. OF GINGIVAL SURFACE LESIONS
Sprague-Dawley	34	12	54	15	22	49
Osborne-Mendel	34	34	252	244	122	140
Holtzman	34	13	60	12	20	52
Black	34	16	76	58	6	37

In this experiment only the Osborne-Mendel strain was consistently susceptible to caries, and the other strains were much more variable. In other experiments involving these four stock strains and also ten inbred strains of rats, consistent differences in the development of caries between strains have been observed.

112. Magnesian Whitlockite in Ashed Dental Tissue—An Identification by X-Ray Diffraction. *Otto R. Trautz, Emma Fessenden, and Margaret G. Newton, New York University College of Dentistry, New York, N. Y.* When dental enamel, which gives the diffraction pattern of apatite, is ignited, the reflections of a new crystal species, whitlockite ($= \beta\text{-Ca}_3[PO_4]_2$) appear in the pattern. From the ratio of the intensities of the whitlockite reflections to those of the apatite reflections in the same diffractogram, one can conveniently estimate the ca/P ratio in the material. Dentin, for instance, after ignition gives a stronger whitlockite pattern than enamel, according to its lower Ca/P ratio. The whitlockite lines in these patterns are shifted in comparison with those of synthetic pure whitlockite, indicating a smaller unit cell. The shift is less in ashed human enamel, greater in ashed human dentin, and still greater in ashed elephant's tusk. This suggests that it is the substitution of Mg for Ca which causes the shrinkage of the unit cell of whitlockite. Pure whitlockite is formed by igniting calcium phosphates with a molar Ca/P ratio of 1.5 at temperatures above 600° C. When heated in the autoclave at 360° C. with steam at 200 atmospheres, it is hydrolyzed into apatite and monetite ($CaHPO_4$). But when fresh elephant's tusk (dentin) is autoclaved under the same condi-

tions, it forms whitlockite from the apatite. We succeeded in preparing magnesian whitlockite in the laboratory at temperatures as low as 100° C. by dripping a very dilute Ca phosphate solution to a Mg phosphate solution. Thus it becomes evident that a Mg substitution in whitlockite lowers its temperature range of formation and stability from above 600° C. to 100° C. or below. This may explain the occurrence of whitlockite in salivary calculi, which A. T. Jensen and M. Danø recently reported. (*J. D. Res.* **31**: 620, 1952.)

113. The Effect of Direct Administration of Sucrose on the Teeth of the Syrian Hamster. *J. F. Volker and C. Schneyer, University of Alabama School of Dentistry, Birmingham, Ala.* It has been previously reported that when mature young hamsters have their first exposure to cariogenic diets at 40 plus days of age, they are most resistant to dental caries. Further information is most desirable and additional investigations are indicated. Thirty-nine hamsters 40 to 41 days of age were selected for study. Groups A and B of fifteen animals each were designated as experimental groups. Group C with nine animals served as a control. All animals were maintained for 100 days on a Purina laboratory diet supplemented with a daily ration of lettuce. Group A animals had 0.1 ounce of sucrose applied to the molar teeth three times each day. Group B animals had one application per day of a similar quantity of sucrose. At the termination of the experiment all animals were examined for dental caries. No significant amount of tooth decay was found in the experimental or control groups. It is concluded that the resistance of these animals to tooth decay is not affected by the direct administration of considerable quantities of sucrose to their tooth surfaces.

114. Fluorine Metabolism in the Albino Rat. *Donovan A. Weddle and Joseph C. Muhler, Department of Chemistry and School of Dentistry, Indiana University, Bloomington, Ind.* Metabolism of small concentrations of fluorine was investigated in the rat. The total amount of diet and water consumed daily and the amount of fluorine excreted in the feces and urine were measured. The difference between the amount of fluorine ingested and the amount excreted represents the amount retained by the animal. One group of animals received a sodium fluoride solution (50 ppm) from the age of 14 to 28 days, while another group received only distilled water. At 28 days of age, one-half of each group received a sodium fluoride solution (5 ppm), and the others received distilled water. The duration of the experiment was 110 days. The animals receiving distilled fluorine-free water and a stock cariogenic diet throughout the experiment stored fluorine at the rate of 1.5 μg F per day per 100 grams body weight. The animals receiving 50 ppm sodium fluoride, and subsequently distilled water, showed a loss of skeletal fluorine during the first 4 to 5 weeks. Following the initial fluorine loss, these animals began to store fluorine at the same rate as the distilled water controls. All animals which received 5 ppm sodium fluoride showed a decreasing rate of fluorine storage, regardless of their previous contact with fluorine, as the experiment progressed. In the previously mentioned instance, the rate was determined as micrograms F stored per day per 100 grams body weight. On a cariogenic corn diet (F conc. = 0.6 ppm) and distilled fluorine-free water, male rats excreted 3.7 μg F per rat per day in the feces and 3.2 μg F per rat per day in the urine. Similar data for the females indicated somewhat less excretion. For the males, this represents 83 per cent excretion (or 17 per cent storage) of the total amount of fluorine ingested, and 75 per cent excretion (or 25 per cent storage) for the females. Similar considerations for the animals receiving 5 ppm F (as NaF) in the drinking water, and the same diet as previously mentioned, indicates that males excrete 12.8 μg F per rat per day in

the feces and 38.9 μg F per rat per day in the urine, while females excrete 15.3 and 313 μg F per rat per day in the feces and urine, respectively. This represents 36 per cent excretion (64 per cent storage) of the total amount of fluorine ingested by the males, and 45 per cent excretion (55 per cent storage) for the females. The animals which received 50 ppm F in the drinking water from 14 to 28 days and subsequently 5 ppm F in the water did not appear to store a different amount of fluorine than similar animals not having previous experience with fluorine.

115. Changes in the Relative Proportions of Oral Bacteria During Topical Application of Penicillin. *Ned B. Williams, Charles F. Eickenberg, and Doris Oshtry, School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* Administration of penicillin topically in the oral cavity to two subjects was undertaken to determine the effects on the relative proportions of nine groups of bacteria isolated from saliva. The subjects are dental students (1) male, aged 23 years, DMF teeth = 18, (2) male, aged 25 years, DMF teeth = 18; diet during the period was essentially identical. Saliva samples were collected on arising over a five-week period; four times in the week preceding use of penicillin; six times per week during the two weeks of use; five times per week for two weeks after use. Potassium penicillin troches (25,000 units) were used three times daily during the second and third weeks. Quantitative estimates of the following groups were made in duplicate: "total" bacteria (I), "total" streptococci (B), "total" gram-negative bacteria (H), *Streptococcus salivarius* (A), lactobacilli (C), fusobacteria (E), Neisseria (F), staphylococci (D), gram-negative bacilli (G), and yeasts (J). No changes in the relative proportions of staphylococci or yeasts were observed during the five-week period. The relative numbers of organisms A, B, E, F, and I were reduced during the administration of penicillin, but gradually increased to prepenicillin levels afterward. Lactobacillus counts were reduced during the first week of application, but returned to prepenicillin levels during the second week even though the antibiotic was in use. The relative proportions of organisms G and H increased steadily during use of the troches. The counts for G reached a maximum during the second week of penicillin application, then decreased. The counts for H reached a maximum after application was stopped and remained relatively constant at this level for the rest of the study. Application of penicillin under the conditions of this study alters the relative proportions of the majority of the groups of microorganisms isolated, and indicates that these procedures can be employed to obtain a better understanding of the balance between certain bacteria in the oral cavity.

116. Polarographic Analysis of Paraffin-stimulated Early Morning Salivary Samples. *Joel Wisotzky, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Salivas were denatured with KOH, precipitated with sulfosalicyclic acid, and the filtrates polarographed in $CoCl_2$–NH_4Cl–NH_3 buffer in the voltage range of –1.4 to –2.0 volts. This procedure was designed to apply the polarographic blood serum cancer test to saliva. This test appears capable of detecting a mucopolysaccharide-like substance in blood, which fluctuates in cancerous and other diseases or inflammatory states. It was felt that similar substances might be liberated by diseased dental tissues into the saliva which bathed them. Thus, a polarographic sampling of the salivas for this class of substances might provide a useful index as to the change in health of the oral tissues. The subjects were part of an intensive experiment, and hence accurate data were obtained with regard to: calculus, hyperemia of gingival tissue, edema of gingival tissue, P.M.A., caries score. Samples for polarographic analysis were taken at the time of plating for

streptococcus and lactobacillus counts, so that the bacterial counts of these organisms in relation to the polarographic picture could be observed. Data thus far analyzed indicate: (1) a similar polarographic effect exists in saliva as in blood; (2) using a paper-weight method for curve area analysis, great area differences between individuals were revealed in voltage range between −1.7 to −1.9 volts; (3) when the area values were large, the tendency toward a low streptococcal count disappeared; (4) no such relationship between area values and lactobacillus count existed.

117. Investigation on the Nature of the Enamel Cuticle. *Jens Waerhaug, Institute of Dental Research, Oslo, Norway.* In young dogs with healthy gingivae, the surface of the enamel (including the enamel cuticle) was ground off from the gingival margin to the cementoenamel junction by means of a thin diamond point. The epithelial cuff (epithelial attachment) was removed or damaged at the same time. After observation periods of three weeks or more the animals were sacrificed and the jaws sectioned. Histologic observations showed that the epithelial cuff was regenerated and of normal appearance. Between this and the ground surface of the enamel there was found a membrane similar to the primary cuticle as far as color, thickness, and microscopic structure were concerned. The primary cuticle represents, according to current opinion, the last cuticular membrane laid down by the ameloblasts. It is supposed to be more highly calcified than the rest of the enamel and of a somewhat different structure. The observations made here do not sustain this interpretation. The possibility may, therefore, be considered that the primary cuticle is not a part of the enamel and that it is not calcified. This explanation is corroborated by the fact that the primary cuticle stains darker than the rest of the enamel when left in the same section. The opposite should have been expected if it had been more highly calcified.

118. Developing Enamel as Seen in the Electron Microscope. *M. L. Watson and J. K. Avery, School of Medicine and Dentistry, Departments of Radiation Biology and Dental Research, University of Rochester, Rochester, N. Y.* Sections of undecalcified hamster lower incisor were prepared and examined by electron microscopy. The inorganic component consists of thin, ribbon-shaped crystals with a length of more than five times the width. The width is between 350 Å. and 450 Å. for most crystals, and the thickness is between 20 Å. and 70 Å. depending on the maturity of the enamel. The crystal thickness increases as the enamel matures. Electron diffraction shows the material to be an apatite. The unit cell *c*-axis is parallel to the long axis of the crystals. The enamel as deposited by the ameloblasts contains crystalline material at all stages of development. The crystals are apparently deposited simultaneously with the organic matrix. There is no apparent cell wall separating the ameloblast or the cytoplasm of its basal process from the forming enamel. It is concluded, therefore, that the deposited enamel is an intracellular product. The three-dimensional form of the enamel rods is complex.

119. Clinical Studies of Dental Caries in Small Groups of Children: Dentifrice, Brushing and Participation Effects. *Paul Weisenstein, Arthur Radike, and Hamilton B. G. Robinson, College of Dentistry, Ohio State University, Columbus, Ohio.* Approximately 150 children, aged 9 to 14, were divided into four groups to determine the usefulness of small groups for caries studies, where careful clinical examinations are supplemented by x-ray examination, bacteriologic examinations, diet studies, and home contacts. Two groups, using a furadroxyl-containing toothpaste, showed the same caries increment as the two groups using the control toothpaste. Two groups who received

instructions to brush after every meal showed 28 per cent fewer new DMF surfaces during the first six-month period than the groups receiving no instruction. All four groups showed 43 per cent fewer new DMF surfaces during the last half than during the first half of the experiment. The average number of new DMF surfaces in all groups during the one-year period was 4.71. In interpreting the caries data, no useful method was found for utilizing the supplementary information to increase the sensitivity of the test. Such information does, however, increase the validity of the experiment. Statistical methods cannot be substituted for accuracy in observations; on the other hand, in this study, extreme thoroughness in the examination procedure did not appear to increase the usefulness of the data for statistical evaluation.

120. Continuation Study of the Effects of Subminimal Amounts of Magnesium on the Syrian Hamster. *George M. Yamane, School of Dentistry, University of Minnesota, Minneapolis, Minn.* A synthetic diet, adequate in all respects except for magnesium, was given to two groups of hamsters. Group I (experimental), consisting of 12 ♀ and 12 ♂, received 40 ppm of magnesium, and group II (control), consisting of 10 ♀ and 13 ♂, received 665 ppm. During a period of eighty days all experimental animals showed hyperirritability, hyperexcitability, marked weight loss, and each experienced one or more convulsive attacks. Grossly no other abnormalities were noted in either group. The lungs, liver, kidneys, femurs, lower incisors and molars, upper molars, heart, and the cecum were sectioned for microscopic study. The vital organs of the experimental animals showed hyperemia with areas of necrosis, particularly in the kidney and liver. In the femur, the trabeculae of the metaphysis were inconspicuous. The bone marrow appeared hyperplastic with a tendency of the white blood cells to shift to the left. The lumina of the sinusoids were narrowed. The dentin and the enamel were malformed as indicated by serrations at the labial aspect of the apical third of the lower incisor. The ameloblasts were oval in shape and were many layers thick (stratified) and the cell boundaries were indistinct. Some of the nuclei of the ameloblasts were pyknotic and karyolytic and many had lost their polarity. There were many basophilic areas (hematoxylin and eosin) of varying sizes in the ameloblastic layer near the apex of the lower incisor. Vacuolization of the cytoplasm of the odontoblasts was present. The stellate reticulum between the inner and outer enamel epithelium was absent. The alveolar bone of the molars was atrophic with very little evidence of remodeling. These findings revealed that the trace element, magnesium, is important in the maintenance and development of certain cells and organs, especially calcifying tissues, of the hamster.

121. Evanston Dental Caries Study. XIII. Preliminary Report on Comparative Fluorine Retention in Human Tissue. *Elizabeth P. Yudkin, Joan Czerniejewski, and J. Roy Blayney, Evanston Department of Health, Evanston, Ill., and Walter G. Zoller Memorial Dental Clinic, Chicago, Ill.* Samples of adult bone, of fetal bone, of blood, and of urine have been collected from persons living in areas using water containing 1.0 ppm fluorine (Area A) and in areas using water containing 0.1 ppm fluorine (Area B). The adult bone samples were taken from the ilium at the point of attachment of Poupart's ligament. The average fluorine content of 12 bones collected from Area A prior to fluoridation was 345 ppm fluorine and of 11 bones collected after 5 years of fluoridation was 651 ppm fluorine (this increased to 706 ppm fluorine after 7 years of fluoridation [12 bones averaged]). Representative bones were collected from 5 fetuses from Area A after 7 years of fluoridation. These compared with samples from nonfluoridated areas showed an average

sevenfold increase in the fluorine content in the femur and in the combined mandible and maxilla and a fivefold increase in the tooth buds. Blood was collected from 42 average individuals living in Area A; the fluorine content of these samples averaged 1.36 ppm. Urine from 33 of these individuals averaged 1.02 ppm fluorine. Blood samples from 11 individuals living in Area B averaged 0.78 ppm fluorine; urine from 10 of these individuals averaged 0.30 ppm fluorine. These are compared with samples of blood and urine from nephritic individuals living in both areas. The blood of 9 nephritic individuals living in Area A averaged 1.70 ppm fluorine; the urine of 8 of these individuals averaged 0.64 ppm fluorine. Nephritic patients living in Area B showed 0.77 ppm fluorine in the blood and 0.32 fluorine in the urine. This is an average of 11 and 10 samples, respectively.

122. Mitotic Activity in the Periodontal Tissues of the Rat Molar. *H. A. Zander and H. R. Mühlemann, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Thirty-one black male rats 5 months of age were studied under standardized circumstances. Mitotic activity in certain periodontal structures was evaluated. Marked mitotic periodicities were noted for the retromolar epithelium and for the periodontal membrane. These mitotic rhythms were compared with the twenty-four-hour rhythms in number of circulating eosinophils and in rectal temperature. The findings seem to indicate that certain oral structures follow the twenty-four-hour periodic pattern of function in the organism as a whole. The effect of a mechanism for mitotic periodicity at the organismic level may be modified by local factors. In the specimens collected at the times chosen for this study, significant differences in the number of mitoses were not detected for the interdental papillae. The role of the presence as well as that of the degree of leukocytic infiltration is considered in this connection. The incidental findings of definite distribution patterns of mitoses within the periodontal membrane and along the molar roots are presented and discussed.

123. Urinary Fluoride Excretion on Exposure to a Fluoridated Drinking Water. *I. Zipkin, R. C. Likins, F. J. McClure, and A. C. Steere, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* In December, 1951, 1 ppm F as Na_2SiF_6 was introduced into the drinking water of Montgomery County, Maryland. Spot urine samples were collected just prior to fluoridation and at 1, 2, 4, 6, 10, 20, and 69 weeks subsequent to fluoridation. The subjects were 5, 6, 7, 8, 12, 13, 14, 30-34, and 35-39 years of age and had no prior history of fluorine exposure. The urinary fluorine value rose slowly in the 5-8- and 12-14-year age groups so that at the end of the 69th week, 0.78 ppm F and 0.85 ppm F, respectively, were found. The 30-39-year-old group reached an apparent plateau value of 1.0 ppm F in the urine after 6 weeks. In January, 1945, 1 ppm F as NaF was introduced into the drinking water of Grand Rapids, Michigan, and spot urine samples were collected at intervals from children 6 to 17 years of age, inclusive. By October, 1952, the urinary excretion of fluorine for each age within this group had reached a maximum value of approximately 0.96 ppm. Similarly, spot urine samples obtained from a comparable age group in Aurora, Illinois, whose municipal water supply naturally contains 1.1 to 1.2 ppm F showed a mean urine content of 0.92 ppm F. For the same time interval the fluorine excretion curves for the three regions are essentially similar. These data indicate that the subjects aged 5 to 14 would be expected to reach a maximum urinary excretion value of about 0.95 ppm F in about 6 years (based upon the Grand Rapids findings). No differences in the excretion of NaF, Na_2SiF_6, and naturally occurring fluoride are apparent.

IV. Abstracts of Papers Presented to the Dental Materials Group.

M-1. Physical Properties of Zinc Oxide-Eugenol Impression Paste. *K. Asgarzadeh, University of Michigan, Ann Arbor, Mich.* Zinc oxide-eugenol impression paste materials are widely used by the dental profession; yet no specification exists for controlling their properties. Furthermore, they are being developed, modified, and used somewhat empirically without a full knowledge of the optimum physical properties that the material should possess. An attempt was made to compare the physical properties of different pastes now on the market, and the properties of eight of the most common ones were investigated. Certain physical tests were made to serve as a basis for comparison. They were mixing consistency, setting time, reproduction of surface detail, and compressive and bend strength. The pastes tested showed measurable differences in these properties.

The primary purpose of this study was not to determine the superiority of one product over another but rather to establish the degree of variation that might be expected between different products. In addition it is believed that this study shows the need for an A. D. A. specification for impression paste materials.

M-2. Effect of Temperature and Molecular Weight on the Water Sorption of Methyl Methacrylate. *G. M. Brauer and W. T. Sweeney, National Bureau of Standards, Washington, D. C.* A detailed study of the water sorption of methyl methacrylate polymers was conducted. At room temperature variations in water sorption of commercial materials are due to the presence of plasticizers and copolymers. Water sorption varies little in the 0° to 60° C. range. Above the second order transition temperature, Tg, sorption increases markedly. No sorption equilibrium was reached on storage of specimens in boiling water for 200 days. At room temperature specimens of different molecular weight show small variations in water sorption. On storage in water above Tg an increase in molecular weight will give a decrease in the amount of water sorbed. Depending on the viscosity molecular weight, water sorption values of the order of two to ten times those found at room temperature were observed for samples stored in boiling water for 200 days. The increase in water sorption at elevated temperature is due to the less rigid linkage between polymer chains and an expansion of the network structure which allows water to permeate more readily into the material. The large increase in sorption above Tg must be considered in processing acrylic materials at elevated temperatures. Crazing and blanching phenomena observed in the processing of dentures may be explained by this phenomenon.

M-3. The Cutting Effectiveness of a Dental Bur as Related to Its Design. *B. Bryton, E. W. Skinner, R. S. Lindenmeyer and R. L. Lasater, Northwestern University, Chicago, Ill.* The study was begun with the defining of the various bur variables, material variables, and experimental variables (operating conditions). These variables were studied in terms of the effect they have upon the magnitude of the cut and upon the life of the bur. This study brings out the need for better methods of analysis than are presently available. Two new theories have been introduced in an attempt to direct the analysis: (a) an empirical mathematical life equation, and (b) a thermodynamic approach to dental bur cutting analysis. The study indicates clearly the need for future experimentation to determine: a suitable experimental material, a method of relating test data on other materials to dentin, the effect that changes in the number of teeth, rake, and clearance angles, tooth surface finish, and edge roughness have upon the cutting action of the bur.

M-4. A Simple Method of Increasing Adhesion of Acrylics to Enamel Surfaces. *Michael G. Buonacore, William R. Wileman and Finn Brudevold, Eastman Dental Dispensary, Rochester, N. Y.* The adhesion of a commercially available acrylic filling material to treated and untreated intact enamel surfaces in the mouth was compared. Tests were performed on eight patients using the labial surfaces of the upper centrals, laterals, or cuspids. All teeth were pumice-cleaned, alcohol-washed, and air-dried. Treatment of the enamel surface consisted of rubbing a drop of phosphomolybdic acid preparation into the surface for twenty to thirty seconds followed by air drying. A drop of 10 per cent oxalic acid solution was then similarly rubbed over the surface and dried. A white coating was formed over the enamel which was washed off with a stream of warm water and the surface was redried with warm air. A small amount of an acrylic resin (about 5 mm. diameter), mixed conventionally, was applied over the treated area and allowed to harden. Controls underwent a similar routine except that water replaced the two reagents. Results showed that the control drops remained on the surfaces for an average of 10 hours as compared to 153 hours for the treated surfaces. All drops on controls fell off by themselves, whereas 90 per cent of the treated drops were mechanically removed at the request of the patient. Of these, 51 per cent were removed within a week of application. In each instance considerable adhesion was present.

M-5. The Reactions of Zinc Oxide With Eugenol. *H. I. Copeland, Jr. (U.S.A.F.) and G. M. Brauer, National Bureau of Standards, Washington, D. C.* Variations in the physical behavior of zinc oxide-eugenol mixtures prompted this study designed to investigate the hardening mechanism of zinc oxide-eugenol. Observation of zinc oxide (reagent grade) and eugenol (U.S.P.) in equimolar mixture indicated that only a small amount, if any, of the two materials reacted chemically under experimental conditions. Over 90 per cent of the eugenol was recovered from such a mixture by chloroform extraction. The pulverized mixture could not be identified by petrographic means. X-ray diffraction examination detected the presence of an additional crystal form besides that of zinc oxide. Zinc oxide of extremely small partical size (125-250 Å) greatly intensifies the reaction. A hard mass formed in approximately four and one-half minutes in a highly exothermic reaction. Examination by x-ray diffraction discloses a much greater amount of the new crystal form. Extraction with chloroform and fractional centrifuging of the extract yielded a sample of higher purity. The physical properties of the crystals, such as decomposition temperature, refractive index, fluorescence, optical activity, etc., have been measured. The chemical composition has been determined. Infrared spectra have been compared with those of zinc compounds of known structure. The experimental results suggest that both stoichiometric and colloidal principles are involved in the setting mechanism of zinc oxide and eugenol.

M-6. Fluid Penetration Between Fillings and Teeth Using Ca^{45}. *W. H. Crawford, University of Minnesota, Minneapolis, Minn.* Twelve teeth with gold and amalgam fillings covering two or more surfaces and regarding which we have a rather complete history were used in this study. The fillings had been in use by patients from several weeks to seven years. The roots were covered with a plastic paint after which the crowns were immersed in a neutralized solution containing radiocalcium (Ca^{45}). The teeth were then embedded in plastic and sectioned through the fillings. After these exposed surfaces were polished, they were placed on sensitized film and radioautographs were prepared. Earlier results using similar fillings which had been

placed in green teeth had shown amalgam to produce by far the least marginal penetration. The teeth in this series, however, showed little difference in penetration between the amalgam filling and the gold inlay. The gingival step in most cases appeared to be the most difficult for good adaptation of filling.

M-7. Effect of Accelerators on the Polymerization Rate of Methyl Methacrylate. *R. M. Davenport and G. M. Brauer, National Bureau of Standards, Washington, D. C.* The effect of amine accelerators in varying concentrations on polymerization kinetics and some physical properties of resulting methyl methacrylate polymers have been studied. The polymerization rate of commercial monomer was measured at 37° C. by the ultrasonic determination of viscosity using the Ultra-Viscoson. This equipment provides a convenient and objective method for observing the polymerization rate by measuring the viscosities from 0.0125 to 50,000 centipoises. To the hydroquinone-inhibited monomer, 2 per cent benzoyl peroxide was added. The effects in different concentrations of over forty accelerators mainly secondary and tertiary amines on the polymerization rate were measured. Wide variations in setting time were observed. In the presence of most secondary and some tertiary amines, no noticeable viscosity change was observed. Short setting times were observed with very few accelerators. The reactivity varied somewhat, depending on the catalyst-accelerator ratio. Only small differences were observed at accelerator concentrations between 1 and 2 per cent. Most polymers formed were highly colored. The polymerization rate followed no simple over-all kinetics, as is usually encountered in the initial stages of the reaction.

M-8. Literature Abstracting and Indexing. *John F. Glenn, The L. D. Caulk Company, Wilmington, Del.* Technical literature is the backbone of research but keeping abreast with publication is a major problem that faces every investigator. A procedure is described to facilitate the preparation of abstracts by the researcher. Two methods of indexing and filing literature abstracts are outlined: (1) through the use of edge-punched hand-sorted cards for the personal file; and (2) the machine-sorted card for institutional use.

M-9. Mechanical Properties of Chromium-Cobalt Alloys Used for Casting Partial Dentures. *L. J. Greves and W. A. Liebfritz (U. S. A.), National Bureau of Standards, Washington, D. C.* The yield strength, ultimate tensile strength, modulus of elasticity, per cent elongation, and hardness were determined on several commercially available alloys. The tensile specimens were rods, 0.09 inch in diameter, having threaded ends $\frac{3}{8}$ inch in diameter, as developed by the School of Dentistry, University of Michigan. The test specimens were cast by the manufacturer and the Central Dental Laboratory of the Walter Reed Army Medical Center. Yield strength (stress at which strain is 1.25 times initial strain) 49,700 to 64,700 p.s.i.; ultimate tensile strength from 91,000 to 116,200 p.s.i.; modulus of elasticity 25.8 to 30.8 X 10^6 p.s.i.; elongation (1 inch gauge) 1.2 to 3.4 per cent; and hardness (Rockwell 30N) 47 to 59. Considerable variation in properties was observed on specimens cast in different laboratories.

M-10. Strain Measurements in Resins. *W. C. Hansen and W. T. Sweeney, National Bureau of Standards, Washington, D. C.* The strain that occurs in polymethyl methacrylate during polymerization is affected by the absorption of water. This effect is a combination of the elongation of the polymethyl methacrylate, due to the swelling caused by the assimilation of a

volume of water, as well as a change in the inherent strain caused by the absorbed water. A mathematical treatment is given the problem so that the change in the inherent strain, exclusive of the swelling elongation, may be determined by the measurement of the water absorbed and the change in total strain. A general description of the procedure followed and the equipment employed is included.

M-11. Some Recent Developments in Dental Hand Piece Design. *E. E. Henry and F. A. Peyton, University of Michigan, Ann Arbor, Mich.* Developmental work at the Physical Research Laboratory of the School of Dentistry, University of Michigan, dealing with (1) ball-bearing hand pieces, made from both steel and light metals; (2) pneumatic turbine type hand pieces, and (3) electric hand pieces was discussed. A ball-bearing contra-angle was discussed also. Approximately two years ago, developmental work leading to a pneumatic turbine type hand piece was undertaken under the sponsorship of the Office of the Surgeon General, United States Army. It was necessary to use ball bearings in this hand piece to allow the instrument to rotate at the usual high speeds. In order to determine the feasibility of ball bearings, conventional type hand pieces were constructed utilizing ball bearings. Later, a small electric motor was added to the ball-bearing hand piece for a motorized hand piece.

M-12. Cutting Characteristics of Dental Burs as Shown by High-Speed Photography. *D. C. Hudson (U.S.A.F.), and J. L. Hartley (U.S.A.F.), National Bureau of Standards, Washington, D. C.* The study of rotating dental cutting instruments is being continued. Emphasis is being placed on securing basic data on the manner in which burs cut different substances. Tooth enamel, dentin, a dentin substitute (resin), Pyrex glass, and a standard brass used by a bur manufacturer were employed. Still photographs with two-millionth-second exposures were made. Action was arrested at speeds of rotation of 5,000 r.p.m.; however, little information as to the true cutting process was revealed. The use of high-speed (3,000 frames per second) motion-picture photography has shown some significant cutting characteristics. The relative effectiveness of steel and carbide burs in cutting human tooth enamel is demonstrated. Dentin, dentin substitute, and brass show curled chips as might be seen in the case of a milling cutter. Glass appears to be pulverized rather than cut, through the repeated blows of the bur blades as the instrument rotates. The technic for making still and motion-picture photographs of rotating burs was presented. The usefulness of these pictures in the evaluation of experimental bur designs was discussed.

M-13. Clinical Evaluation of a Resin Cement. *J. F. Johnston, Indiana University, Indianapolis, Ind.* Approximately 350 crowns and bridges have been set using this cementing medium. This paper covers the clinical evaluation at the end of a one-year period. The paper included a discussion of the material; the necessary differences in the preparation of the field; the technique and the variables affecting the technique; the failures and the reasons for them; and the advantages and disadvantages.

M-14. Dimensional Stability of Self-Curing Acrylic Resin Dentures. *P. M. Jones and E. W. Skinner, Northwestern University, Chicago, Ill.* The curing shrinkage and dimensional changes during storage in water of a self-curing denture acrylic resin have been studied under various conditions of technical procedure. The effect of temperature, time in the flask, molding, mold lining, and similar factors were included. The fit of the denture was estimated both by visual inspection and by measurement between gauge marks on the stone cast and on the denture base.

M-15. Direct Resin Filling Materials: Coefficient of Thermal Expansion and Water Sorption. *J. Lal, R. Green, J. Cornell and E. E. Rose, H. D. Justi & Son, Philadelphia, Pa.* The coefficient of thermal expansion of polymethyl methacrylate resin decreases with the addition of various inorganic fillers; a reduction in the increase in weight due to water sorption of the resin also takes place with the addition of fillers. The greater the amount of the filler added to the direct filling resin, the greater is the reduction in the coefficient of thermal expansion and gain in weight due to water sorption. Addition of cross-linking material to the direct filling resin does not significantly change the water sorption properties of the resin; change of pH likewise has no noticeable effect on water sorption.

M-16. The Water Added Technic for Hygroscopic Expansion. *D. B. Mahler, University of Michigan, Ann Arbor, Mich.* A new technic for inlay casting compensation is described whereby the hygroscopic expansion of a dental casting investment is controlled by adding specific quantities of water. Practical and theoretical evidence indicates the need for different compensations for different types of inlays and crowns. This new technic allows for the desired change of investment hygroscopic expansion. In addition, the ability to reproduce a given expansion value is demonstrated. The equipment necessary for this technic is also described.

M-17. Some Factors Affecting the Dimensional Stability of the Ag-Sn-(Cu-Zn) Amalgams. *J. A. Mitchel (U. S. Navy), I. C. Schoonover, G. Dickson and H. C. Vacher, National Bureau of Standards, Washington, D. C.* An investigation of the x-ray diffraction characteristics and of the dimensional changes of Ag-Sn-(Cu-Zn) amalgams during and after their hardening period indicated that uncombined mercury was present in the amalgams after the initial solidification and that this uncombined mercury disappeared during the period when greatest dimensional changes occur. The presence of uncombined mercury was determined by studies of x-ray diffraction patterns produced by the crystalline structure of mercury when the amalgam was cooled to –125° C. The dimensional changes of the amalgam specimens were observed by means of dental interferometers at room (21° C.) and body (37° C.) temperatures under standardization procedures. On the basis of the data obtained it is postulated that the expansion during the hardening of amalgam results from diffusion of uncombined mercury throughout the material; the subsequent shrinkage then results from combination of this mercury with existing phases or with residual alloy.

M-18. Effect of Temperature on Flow of Alginate. *F. H. Nealon.* A study was made of a physical property common to all nonreversible hydrocolloids in varying degrees. Materials purchased at dental supply depots were weighed and mixed at different temperatures using a standardized procedure. They were then placed in equipment which duplicates the constant temperature and humidity of the mouth. This equipment permits an observation of the flow in one direction. The duration of the flow was recorded. The amount of flow was ascertained by weighing the displaced mass. The ratio of the amount of flow to the total weight was recorded as per cent of flow. Results are in graph form. An amount of flow is necessary to reproduce undercut areas and minute detail. The flow of alginates, however, is opposed to the flow of plaster, its predecessor. Plaster was placed in the mouth when the flow had practically ceased. Alginates start to flow when they are placed in the mouth when they are used as suggested, and in the customary manner. The flow, as a property of the alginates, may have some effect on the accuracy of reproduction of the palatal surface if the impression technic is not adapted

to restrict, diminish, or confine the flow. It could be of no consequence in lower impressions because the flow would be into the area to be duplicated. Extensive research reports confirm the accuracy of the alginates for impressions of cavity preparation. When used in this manner, the material is locked into the cavity and flow is not active. Flow varies with temperature.

M-19. Hazards of Dental Radiography. *R. J. Nelson and G. C. Paffenbarger, National Bureau of Standards, Washington, D. C.* A 16 mm. sound and color motion picture, entitled "Hazards of Dental Radiography," has been completed. The film illustrates the elementary physics concerned with dental radiography and by showing actual radiographic procedures in the dental office points out the radiation hazards attendant to the use of x-radiation by the dentist. Recommendations are made which will assure the dentist following them that all personnel in and adjacent to his office will not be subjected to dangerous levels of radiation. The recommendations are based upon those outlined in Handbook 41 of the National Bureau of Standards.

M-20. Application of Ultrasonics to the Cutting of Teeth. *A. G. Nielsen and J. Richards, United States Naval Dental School, Bethesda, Md.* The phenomena of magnetostriction and piezoelectricity have been well known for some time, but only recently have these effects been used combined with dies and abrasives to cut hard materials. The possibility of utilizing these procedures in dental clinical situations was realized by the Naval Dental Corps. Subsequently the Bureau of Medicine and Surgery, United States Navy, approved a project inquiring into the possibility of developing a dental hand piece operated at ultrasonic frequencies. The project included three phases: (1) the development of a hand piece operating on the afore-mentioned principles; (2) the observation of the biologic effects of the use of such an instrument; and (3) the application of such instruments in the dental clinic. Efforts in phase 1 of the project are being directed toward solution of the mechanical problems related to the design, construction, and operation of a hand piece utilizing the afore-mentioned phenomena. Initial efforts in phase 2 include a series of cutting tests which were made on the teeth of two dogs. Instruments were used which included hand pieces utilizing (1) a piezoelectric driver; (2) a high-speed diamond wheel; and (3) a magnetostriction driver. Progress to date has been such as to arouse hopes that clinical application of some models may soon be accomplished. Developments in all three phases of the project will be reported.

M-21. Resin Cements and Direct Filling Resins for Posterior Teeth. *G. C. Paffenbarger and H. J. Caul, National Bureau of Standards, Washington, D. C.* The consistency of four mixed but unhardened resins was determined with a test similar to those employed in A. D. A. specifications for zinc-phosphate and for silicate cements. A standard consistency was selected that required from 1.0 to 1.3 grams of polymer (powder) to 0.5 milliliter of monomer (liquid), depending upon the trade brand. Most tests were conducted on specimens of standard consistency. The times of hardening fell within a narrow range of $5 \pm \frac{1}{2}$ minute. Even a wide variance from standard consistency extended the range little ($5\frac{1}{2} \pm 1$ minute). The resins are less critical in this regard than are the zinc-phosphate cements. Film thickness varied from 115 to 30 μ, depending upon the consistency and the trade brand. Usually the thinner the consistency, the lower the film thickness. The rise in temperature above mouth temperature during polymerization ranged from 13° to 29° C.; values for zinc-phosphate and silicate cements were 2° to 22° C. The compressive strength of the resins was approximately that of the zinc-phosphate cements, 12,000 ± 1,000 lb. per square inch. Opacities varied from

100 to 40 per cent among the trade brands and among the colors of some brands. The solubility of the resin compounds was near that of zinc-phosphate cements using the test in A. D. A. specification No. 8 for zinc-phosphate cement. No significant amounts of arsenic were detected on composite samples of all colors of each resin. Other physical and chemical properties are being measured.

M-22. Appearance of Tooth Cavity Walls When Shaped With Various Instruments. *F. A. Peyton and J. F. Mortell, Jr., University of Michigan, Ann Arbor, Mich.* This report described the surface conditions developed on the cavity walls of teeth cut with various dental instruments. The procedure employed to observe these conditions was explained, and details of the technic described. The results obtained from the use of various burs, diamonds, and hand instruments were presented, together with the observations of surface conditions resulting from both wet or dry surfaces and high or low speeds of operation with rotating instruments.

M-23. Further Studies on the Electrical Conductivity of Dental Cements. *L. J. Phillips and R. W. Phillips, Indiana University, Indianapolis, Ind.* This study is a continuation of previous research dealing with measurement of the actual electrical conductivity of dental cements. The report covers the influence played by moisture upon the electrical conductivity; the resistance of the cement is markedly reduced when exposed to normal saline solution. Cells of varying thickness and contacting surface area have been tested to determine the influence of these variables. The effect of temperature upon the conductivity has been evaluated. Cells employing gold castings cemented onto a full crown preparation were employed in certain tests to simulate more closely the clinical conditions. Various cementing materials are being compared for their insulating characteristics: zinc phosphate, resin, calcium hydroxide, zinc oxide, and eugenol. These materials have been tested under both wet and dry conditions.

M-24. The Screening of Materials for Adhesion to Human Tooth Structure. *E. E. Rose, J. Lal, N. B. Williams and J. P. Falcetti, H. D. Justi & Son, Philadelphia, Pa.* More than 5,500 individual tests for the adhesion of various materials to tooth structure were conducted. There is a difference in the adhesive qualities when materials were used against enamel as compared to dentin. The behavior of adhesives when tested on dry teeth has no direct bearing on the potential adhesion of the same material in the presence of moisture. The addition of fillers shows promise toward improving adhesion by better wetting of tooth surface and a potential decrease in the thermal coefficient of expansion of resins. Water sorption by the resin reduces or destroys the initial adhesion of the material to the tooth structure. None of the commercial resin filling materials tested consistently maintained adhesion after immersion in water. The data also indicate that a test bacterium can lodge between the filling materials employed and the tooth cavity when teeth are maintained in a culture at 37° C. Effects of temperature variation could not accurately be evaluated. Addition of a filler to an adhesive copolymer did not reduce the chances for lodgment of the test bacteria. The test methods employed in development of data for the paper were described.

M-25. A Study of the Use of Irreversible Impression Materials in the Sears Technic. *E. W. Skinner and F. B. Carlisle, Northwestern University, Chicago, Ill.* Hydrocolloid impressions were made of a three-tooth bridge model fitted with gauge marks in critical areas. A comparison of the distance between the gauge marks as reproduced in the impression with the original

distances on the model was taken as an indication of the accuracy of the impression. The accuracy of impressions obtained with six commercial irreversible impression materials was tested in this manner with twelve different variations in technic. The accuracy of impressions obtained with three reversible impressions was tested likewise. It was found that the gelation time of the irreversible materials should be increased to six minutes from the average time of four minutes at room temperature if an accuracy equal to that of the reversible materials is obtained under simulated mouth conditions. Such a delay in gelation time can be attained if ice water is employed for mixing the irreversible material. However, a greater accuracy was obtained than with any of the reversible materials in one case where the retardation was effected with chemicals. No advantage was gained by increasing the water/powder ratio. Under the experimental conditions employed, the use of a perforated tray provided better accuracy than when an individually constructed nonperforated tray was employed.

M-26. Gallium Alloys. *D. L. Smith, National Bureau of Standards, Washington, D. C.* The substitution of gallium for mercury as a liquid metal in the formation of plastic alloys has been suggested. The physical properties of alloys in which gallium is a major constituent have not been determined. Literature surveys reveal the paucity of such information, the determination of physical constants of gallium, some phase diagrams, and the effect of trace amounts of gallium on the physical properties of other alloys being the primary objects of prior research. At room temperature gallium was found to alloy most readily with powdered metals having a face-centered cubic structure such as nickel, gold, platinum, and silver. Alloying did not take place readily with powdered metals of the body-centered cubic system such as chromium, molybdenum, and tantalum. Preliminary determinations of compressive strength and hardness of some alloys of gallium and nickel gave strengths of the order of 100,000 p.s.i. and Brinell Hardness Number of the order of 100. Binary and ternary alloys containing gallium and powdered metals have been made which were plastic and then hardened at room temperature. After hardening, these alloys did not melt at several hundred degrees above room temperature.

M-27. Repair of Dentures. *J. W. Stanford and C. L. Burns, National Bureau of Standards, Washington, D. C.* The ability of both heat-curing and self-curing resins to make a satisfactory repair was determined using the transverse test as specified in A. D. A. specification No. 12 for denture base resin and in determining the dimensional changes occurring in dentures repaired with a variety of materials and technics. Repairs made with self-curing repair resins had approximately 60 per cent of the original strength of the denture base resin, while repairs with the heat-curing type were approximately 85 per cent as strong. Dentures repaired at 100° C. had two and one-half times the amount of dimensional change as dentures repaired at 73° C. Dentures repaired with self-curing resins were approximately 0.003 inch (0.18 per cent) wider than the molar-to-molar dimension on the unbroken denture. When repairs were effected with the heat-curing resins at 100° C. the change was in the opposite direction 0.013 inch (0.80 per cent). At the 73° C. cure the change was 0.005 inch (0.31 per cent). Therefore, the heat-curing resins when polymerized at 73° C. make the most satisfactory repair.

M-28. Air-Driven Dental Hand Piece. *P. H. Tanner and R. C. Mitchell, U. S. Naval Dental School, Bethesda, Md.* Recent investigations have shown the advantages of instruments utilizing increased rotary speeds for the cutting of teeth. The possibility of using air as a power source to achieve high-speed

rotation was realized by the investigators. Research is being conducted at the United States Naval Dental School (Bethesda) to design an air-driven dental hand piece. The development of such a hand piece is being conducted in two phases. Efforts in phase 1 of the project are being directed toward solution of the mechanical problems related to the design, construction, and operation of a hand piece utilizing the afore-mentioned power source. Phase 2, the clinical application of the afore-mentioned instrument, awaits completion of phase 1. Current models of the air-driven hand piece have achieved rotary speeds of up to 55,000 r.p.m. Progress to date has been such as to arouse hopes that clinical application of some models may soon be accomplished. Development to date will be reported.

M-29. Artificial Dentures and the Oral Mucosa. *G. Van Huysen, W. Fly and L. Leonard, Indiana University, Indianapolis, Ind.* The toleration of most oral mucous membranes to denture bases has been demonstrated over the years by a considerable clinical experience. However, some artificial dentures are on occasion found associated with pathologic tissue reactions. In other words one can never unconditionally guarantee the success of a prosthetic appliance any more than one can guarantee the success of therapeutic treatment. This is true because living tissues differ in different individuals and are not static but may and do sometimes change over a period of time. This study describes the clinical and histologic appearance of four types of lesions of oral mucous membranes associated with artificial dentures. There is also a discussion of the cause and treatment as well as classification of these lesions.

M-30. An Objective Study of the Effect of Manipulative Variables on the Properties of Dental Amalgams. *A. L. Ware and A. R. Docking, Bureau of Dental Standards, Australia.* Even under the application of apparently rigid control, present tests on dental amalgam specimens prepared by hand are found to be subject to marked personal variation. In order to minimize this personal factor, a pestle and mortar has been mechanized, and a variable-load mechanized plugger capable of condensing an amalgam in layers according to a definite pattern has been constructed. The use of these machines in accurately determining the effect of trituration load, pestle revolutions, and condensation load on the expansion of amalgams is demonstrated, since this test was considered the most sensitive. The conclusions reached from this series of tests are: (1) the construction of machines to control the manipulative variables has placed the testing of dental amalgams on a more scientific basis; (2) alloys differ considerably in their reaction to variations in trituration load and number of pestle revolutions; (3) relatively, trituration variables have a far greater influence on the expansion of an alloy than condensation load; (4) within limits, the consistency and dimensional change of an amalgam are both directly dependent on the trituration "work" taken as the product of the load and pestle revolutions; (5) for comparable mixes, the expansion of an amalgam is not appreciably altered by using a mechanical amalgamator instead of mortar and pestle.

M-31. A Study of Factors Affecting Adhesion of Acrylic Autopolymers to Tooth Structure. *William R. Wileman, Finn Brudevold and Michael G. Buonacore, Eastman Dental Dispensary, Rochester, N. Y.* Flat dentin and enamel surfaces of human teeth embedded in plaster were used. The area of adhesion was limited by aluminum foil with a 5 mm. diameter hole punched in it which was weakly held in place on the tooth surface by mucilage. Special matrix rings confined the acrylic to this area and allowed easily adaptable vertical force to be applied for the adhesion test. Fourteen

resins were tested under different conditions. Results show the following: 1. The presence of moisture, (a) prior to application of the resins and (b) after a bond is obtained, will reduce or entirely prevent bond formation. 2. Increased surface roughness increases the adhesion to dry surfaces. 3. Temperature interchanges (9° to 55° C.) decrease the adhesion of acrylic to glass surfaces (coefficient of thermal expansion same range as tooth structure). Glass surfaces were used to avoid the presence of moisture from the tooth. For the same reason, temperature changes were obtained by using an oven and refrigerator in preference to water baths. The clinical implications of these results are: (a) adhesion is directly related to the degree of dryness of the tooth surface, at the time of placement of the resin; (b) acrylic-tooth bonds, if obtained clinically, are quickly destroyed by oral fluids; (c) temperature changes in the mouth are capable of weakening acrylic-tooth bonds.

V. Abstracts of Papers Read by Title

T1. Effects of Ovariectomy on the Periodontal Tissues. *R. Gordon Agnew, H. Henry Weisengreen, and Russell D. Coleman, College of Dentistry, University of California, San Francisco, Calif.* This report deals with histologic findings in the jaws of an animal of approximately 6 years of age which had been ovariectomized for one year and five months. Microscopic sections, mesiodistal and labiolingual, including gingivae and alveolar bone, were examined and compared with control tissues. No outstanding or consistently observed histologic findings were observable. Some evidence was noted of changes in the prickle-cell layer, e.g., marked vacuolization of cells with nuclear displacement or disappearance. There was no evidence of the breakdown of connective tissue, which is commonly noted as part of the initial stages of human periodontosis. There was some evidence, not, however, consistently seen, of excessive osteoclastic activity. No significant cemental changes were detected. Some evidence of increased vascularization of the periodontium was observed, but was not a consistent finding. In the roentgenographic observation of the jaws of all animals studied in the project there is again no evidence to date of diagnostically significant bone changes following bilateral ovariectomy. Further histologic studies are in progress.

T2. Primary Inductions of Tooth Formation. *James K. Avery, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Extirpation of selected areas of neural crest of *Amblystoma maculatum* at the time of neurulation (stage 15) resulted in a corresponding lack of tooth formation in the jaws. Homoplastic transplantation of similar areas of neural crest tissue to the abdominal wall of host animals resulted in normal tooth formation at this site. Moreover the abdominal wall epidermis was induced by the underlying neural transplant to invaginate and form the enamel organs of the teeth. It is consequently surmised that the neural crest cells function in the primary induction and formation of the tooth anlage in this species.

T3. Post-Eruptive Maturation of Teeth. 1. Radioiodine Uptake in Ivory. *Julian D. Boyd and Alvin S. Blum, Veterans Administration Hospital, New Orleans, La.* This is the first of a series of studies designed to provide methods for the quantitative comparison of post-eruptive changes in dentin in different teeth. The present study deals with the degree and manner of uptake of radioiodine (I^{131}) in vitro by small thin slabs of ivory from an elephant tusk. Findings to date indicate that: (a) in terms of unit weight of substance, each slab of ivory when immersed in a solution of I^{131} for successive periods of time will take up the radioisotope at a rate peculiar to it-

self, in comparison to other slabs cut from the same general region; (b) the degree of uptake will vary from slab to slab, and within the substance of any given slab, in proportion to the relative permeability of the respective area; (c) the uptake is conditioned by factors other than or in addition to "water space"; (d) zonal differences in uptake can be demonstrated by radioautograph; the zones of differential uptake correspond to histologically distinguishable areas of dentinal structure.

T4. Effect of Certain Anti-enzymes on Acid Production in Plaque. *Finn Brudevold and Marguerite F. Little, Eastman Dental Dispensary, Rochester, N. Y.* Subjects were instructed to brush their teeth and rinse with solutions of sodium lauroyl sarcoside in the morning upon rising and before going to bed at night. After this procedure had been used for seven days the fall in pH of plaque material from proximal surfaces following a sugar rinse was measured, using the technic of Campaigne (*Ill. D. J.* **10**: 85, 1941). The tests were performed in the morning nine to twelve hours after the last brushing or rinsing. Similar tests were performed after the subjects had been using toothpaste containing sodium lauroyl sarcoside and dehydroacetic acid for one week. All tests gave negative results. In other experiments the inhibitory effect of single mouth rinses of lauroyl sarcoside was studied. Using an intraoral technic for measuring pH, it was found that this compound exerted an inhibitory effect on the fall in pH after one-half hour. A decreased acid production was also found in salivary specimens obtained one, two, and three hours after a lauroyl sarcoside rinse. On the basis of these findings, it was concluded that lauroyl sarcoside does not reduce the acid production in subsurface material of bulky plaque.

T5. Periodontal Fiber Reattachment in Replanted Incisors of the Monkey. *Earl O. Butcher, College of Dentistry, New York University, N. Y.* To see to what extent periodontal fiber reattachment would occur under very adverse conditions in the incisors of the Rhesus monkey, extracted teeth from which the organic material and superficial dentin had been burred were replanted in their original alveoli. Extracted teeth were also immersed in 10 per cent formalin, alcohol, or boiling water prior to replantation. After a lapse of 20 to 120 days, the monkeys were sacrificed and the jaws were histologically prepared. Reattachment of fibers occurred in most cases, after such treatment, by the formation of new layers of cementum or bone, in which the free ends of the periodontal fibers were engulfed. Areas of ankylosis and resorption were common along the root surface of the replanted teeth. Infected areas were mainly confined to the apex and the sulcus. Of the various treatments employed, prior to replanting, infection was less common while ankylosis was more frequently encountered in the teeth treated with formalin and alcohol. Resorption was greatest in teeth treated with formalin. Where infection existed there was no reattachment of the periodontal fibers. Epithelial pegs and downgrowth along the root were closely associated with the infection and lymphocytic infiltration in the sulcus. These investigations indicate that while infection does inhibit reattachment of the periodontal fibers, epithelium spreads over the exposed surface of the infected area.

T6. The Latin Square Design in Clinical Experimentation. *Neal W. Chilton, Faculty of Medicine, Columbia University, New York, N. Y.* The latin square design, which has been used widely in agricultural experimentation, is presented as a design for clinical research. It can be used to control two factors while studying the effect of a third factor, such as treatment. This design is explained, and an example is presented in which four drugs and a placebo were studied for their effectiveness in making cerebral palsy patients

more cooperative for operative dental procedures. The latin square design allowed for the assessment of variation due to the different order or sequence of operative experience, due to the different patients employed, and due to the different drugs. The evaluation of the results by the analysis of variance is presented in detail.

T7. A Method of Scoring Alveolar Bone Resorption in Hamsters. *Emmett R. Costich, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* A graph similar to that used in recording pocket depth in human beings was devised to show the crown and roots of the hamster molars. By visual examination the height of the alveolar crest in stripped jaws was recorded. Frosted acetate paper was then placed over the charts and the area between the cementoenamel junction and the alveolar crest traced, cut out, and weighed. The weight of the paper was then regarded as a score of bone loss. Such scores permit critical evaluation of the observed changes. By this method, it was observed that alveolar crest resorption is most prevalent on the lingual surfaces of the first molar teeth in both jaws. This is in contrast to caries, which occurs most frequently on the buccal surfaces of second and third molars. Castration of males reduced the alveolar crest resorption, while ovariectomy in females increased it.

T8. The Effectiveness of Sodium Fluoride, Sodium Monofluorophosphate, Copper and Chlorophyll Derivatives in Reducing Alveolar Crest Resorption in the Hamster. *Emmett R. Costich, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* In view of a recent report on the importance of certain bacterial enzymes in gingival disease (Schultz-Haudt, Bruce, and Bibby, *J. D. Res.* **32**: 681, 1953) and the reports of gingival disease in the Syrian hamster (Keyes and Likens, *J. D. Res.* **25**: 166, 1946; Mitchell, *J. D. Res.* **29**: 732, 1950), the jaws of hamsters raised on caries-producing diets and drinking water containing 40 ppm NaF, 40 ppm $NaFPO_3$, 50, 25, and 10 ppm of Cu^{++}, and various 0.2 per cent water-soluble chlorophylls were scored for alveolar crest resorption. Copper was most effective, producing statistically significant reductions in both sexes at 50 and 25 ppm. NaF and $NaFPO_3$ produced statistically significant reductions in the males. One chlorophyll compound (93.2 per cent pure with high labile copper content) in the drinking water produced a statistically significant reduction in alveolar crest resorption in both sexes. Magnesium, iron, and two copper chlorophyllins (75 per cent and 94.4 per cent purities) failed to produce any change in alveolar crest resorption.

T9. Major Variant Dimensional Proportions of Tooth Components Are Viewed as Attributes in Genetic and Morphologic Studies of the Dentition. *Albert A. Dahlberg, Department of Anthropology, University of Chicago, Chicago, Ill.* A related series of genetically determined major variants pertaining to dimensional proportions of certain tooth components have been observed in human dentitions. Similar conditions have been produced in certain gene combinations in experimental animals. Human pedigrees show these traits to be inherited as a simple dominant. A good descriptive name for the trait is still wanting. There are four specific exhibitions of this character. They are: 1. In the premolars: Marked decrease of the buccolingual and marked increase of the mesiodistal measurements making the tooth appear as though it had been compressed physically. It is almost exclusively a trait seen in the second premolar. 2. In the lower deciduous first molar: Marked decrease of the buccolingual dimension of the anterior portion of the occlusal surface without decrease in the comparable coronal measure. This gives the tooth a triangular-appearing occlusal surface rather than the typical oblong

appearance. 3. In the deciduous second molar: Deciduous second molars differ from the permanent first molars in size and proportion of the occlusal surface measurements. The occlusal markings are almost identical in other respects. The deciduous second molar occlusal surface is longer mesiodistally in proportion to the breadth than is the permanent first molar. 4. In all posterior teeth: Individual and population differences exist in the proportion of the buccolingual measure of the crowns of molar and premolar teeth as compared to the similar measure between the buccal cusps and the lingual cusps. This is a test of available functional surface of the occlusal surface as compared to the bulk dimension of the tooth. These four entities have in common the variation in proportion of tooth components and are significant in genetic and morphologic studies because they indicate certain trends and characteristics of a given dentition. Pedigrees show the inheritability of these attributes.

T10. Margoid Differentiation of the Tuberculum Dentis. *Th. E. De Jonge, Department of Anatomy, University of Amsterdam, Amsterdam, Netherlands.* Bilateral symmetrical margoid differentiation of the tuberculum dentis of maxillary canines, revealing the universal character of this anomaly, is described. The manifestation occurs in all dental elements that should be considered—the first and second incisors and the canines.

T11. Multiple Hyperodontia in Upper and Lower Jaw. *Th. E. De Jonge, Department of Anatomy, University of Amsterdam, Amsterdam, Netherlands.* Multiple hyperodontia is characterized and distinguished by the fact that in each of the four groups of teeth, the incisors, the canines, the premolars, and the molars, the typical number was exceeded.

T12. L-Nordefrin. *Edward C. Dobbs, University of Maryland Dental School, Baltimore, Md.*—L-Nordefrin (levo-cobefrin) is an isomer of epinephrine and is prepared synthetically. Its hydrochloride salt is water soluble, and it is levo-rotatory thereby differing from cobefrin, which is a racemic mixture. In a 1:20,000 solution it has comparable vasoconstrictor properties with cobefrin 1:10,000, and when combined with procaine (2 per cent) it has a similar duration time of anesthesia symptoms. L-Nordefrin (l-cobefrin) appears to be an effective vasoconstrictor for use in local anesthetic preparations.

T13. Intracellular Oxidative Enzymes in Oral Tissues Following Exposure to High Intensity X-Radiation. *Bertram Eichel, USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.* To date there is no reliable criterion available for determining the extent of early damage to the mammalian organism following exposure to high intensity x-radiation. It has, therefore, become apparent that it is necessary to establish some tissue or tissue complex which could be used as a biologic dosimeter. Tissues such as gingiva and tooth pulp, which may be readily biopsied and which are dispensable when contrasted with the many indispensable tissues of the body, appear particularly attractive as possibilities. As a working hypothesis it was assumed that sufficiently high dosages of x-radiation might cause uniform patterns of metabolic upset in all tissues. Rabbits were, therefore, subjected to high intensity x-radiation dosages applied locally to the head. Twenty-four hours after exposure the animals were decapitated and ten tissues were immediately removed. One was removed from the abdomen and nine were taken from the head including gingiva, tooth pulp, and sublingual gland. Cytochrome oxidase, succinic dehydrogenase, DPN cytochrome c reductase, catalase, glucose-6-PO_4 dehydrogenase, and total protein were assayed in each tissue homogenate. Preliminary data thus far obtained tend

to substantiate the working hypothesis that inhibition of succinic dehydrogenase and cytochrome oxidase occurs in all tissues studied. Parallel histopathologic examination of the dental tissues was negative. At present an intensive program is under way to extend these preliminary experiments.

T14. Long-term Observations of Radiation Changes in Salivary Glands and the General Effects of 1,000 r to 1,750 r of X-ray Radiation Locally Administered to the Heads of Dogs. *James A. English, Merrill G. Wheatcroft, H. W. Lyon, and Clarence Miller, Naval Medical Research Institute, Bethesda, Md.* Considerable morphologic alteration was observed in the salivary glands of dogs which had received 1,750 r of 200 KV x-ray radiation locally to the head. From a total of six dogs which were irradiated, five were sacrificed during a five- to sixteen-month period following exposure and provided the experimental material for histologic study. Normal glands were obtained from seven unirradiated dogs for comparison. Several special fixatives and stains were used in order to adequately observe the nuceli, mitochondria, connective tissue, and various cellular products of the salivary glands. The parotid, submaxillary, and sublingual glands of three dogs which received 1,750 r and were sacrificed 5¾, 6½, and 8 months later were markedly atrophied, fibrosed, and altered in morphologic detail. Except for knowledge of the anatomic areas from which the tissues were taken, or by special staining reactions, it would have been impossible to identify many tissue sections. The severe changes consisted in various grades of obliteration of acini, leaving whorls of nuclei in streaked concentric patterns, fragmented cells, groups of cells without pattern, partly damaged acini in which one or two cells were identifiable, and the rest being vacuolated or in signet-ring configuration. A dog receiving the same amount of treatment, which was sacrificed at 14¾ months afterward, had much less apparent total damage, but in many areas some of the changes described previously could be found. A dog receiving 1,000 r and sacrificed 15½ months afterward had relatively normal-appearing salivary glands. The general changes observed were stomatitis; surface sloughing and oral hemorrhage in mild to moderate degree; erythema of exposed areas, particularly over the ears and around the eyes; and epilation. Hair grew back in after six to twelve weeks and was light gray in color. Dogs appeared to be moderately distressed after treatment, and one animal developed an ulcerating lesion of the neck.

T15. Patterns of Correlation in Tooth Developments in the Frog. *Roy Gillette, Departments of Histology and Oral Pathology, University of Illinois, College of Dentistry, Chicago, Ill.* The development of replacing teeth was studied in twenty-four frogs of various ages after metamorphosis. The teeth-bearing bones were removed with teeth, tooth germs, and periodontal tissues intact, were stained with alizarin red S in 95 per cent alcohol, and were cleared in oil of wintergreen. Alizarin stained the enamel and dentin undergoing calcification at the time of staining, while the remaining tissues were transparent. On the basis of staining pattern, more than twenty different steps could be distinguished in the cycle of tooth development from germ initiation through eruption and resorption. A precise correlation between the eruption of a tooth and the initiation of its replacing germ was observed. There was also a correlation between the development of a given tooth and that of its neighbor on either side. If the entire cycle be divided into four stages of equal length and a given tooth is in Stage 4, then its replacing germ will be in Stage 2, the neighboring teeth in Stage 3, and their replacing germs in Stage 1. The causal mechanism responsible for this pattern is postulated as an inhibition exerted by tooth germs in Stages 1 and 2 upon the dental lamina, preventing the formation of other tooth

germs in their immediate neighborhood. L. Bolk explained the alternating patterns he observed in reptiles by postulating different origins for "endostichial" and "exostichial" families of teeth, whereas these are identified in the present study as Stages 1 and 2, respectively, in a single continuum of development.

T16. Modification of the Effect of Cortisone Upon Alveolar Bone by the Systemic Administration of Estrogen. *Irving Glickman and Gerald Shklar, Tufts College Dental School, Boston, Mass.* Osteoporosis of alveolar bone has been reported in mice subjected to daily injections of cortisone. The present experiment was conducted to determine the manner in which the aforementioned cortisone effect upon alveolar bone would be influenced by systemic administration of estrogen. Sixty male and female young adult albino mice, weighing approximately 20 grams each, were used as experimental animals. Forty were injected daily with 0.25 mg. of cortisone. Twenty served as controls. Of the forty cortisone-injected animals, twenty received an additional weekly injection of 600 R.U. of estradiol benzoate. The duration of the experimental period was five weeks. In the animals injected with cortisone alone, microscopic examination of the alveolar bone revealed osteoporosis characterized by a marked diminution in osteoblasts and osteoid formation. In the animals receiving estrogen in addition to the cortisone, there were some areas of alveolar bone in which bone resorption was noted. However, new bone formation characterized by the presence of osteoblasts and notable amounts of osteoid was prominent along the endosteal margins and along bone in relation to the periodontal membrane. In general, the bone changes were more marked in the females.

T17. Ribonucleic Acid Content of Human Gingiva. *Wesley Halpert, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* As part of a study on the etiology of periodontal disease, ribonucleic acid was determined in human gingiva. Tissue sections were first treated with desoxyribonuclease to remove desoxyribonucleic acid. The sections were then stained with azure A. The quantities of ribonucleic acid present in the nucleoli of the gingiva were measured by absorption microspectrophotometry as previously reported for desoxyribonucleic acid (*J. D. Res.* **32**: 699-700, 1953). To eliminate as many errors as possible, four pieces of tissue, namely, one normal, one from a case of periodontosis, one from a case of periodontitis, and one rat liver were embedded in each paraffin block. Therefore, comparisons of the relative amounts of ribonucleic acid in these tissues were made on the same slide. Nucleoli from the basal layer of the epithelium of ten periodontosis, ten normal, and ten periodontitis specimens have shown wide variation in ribonucleic acid content and no correlation between ribonucleic acid content and the presence or type of periodontal disease.

T18. Comparative Effects of Two Basic Diets With and Without EDTA on Experimental Caries in Rats. *M. Rachel Harris and Robert M. Stephan, National Institute of Dental Research, Bethesda, Md.* It has been found that the addition of EDTA (ethylene diamine tetra acetic acid) increased caries in rats fed fine particle diet 580 (Stephan and associates, *J. D. Res.* **31**: 475, 1952) and also coarse particle diet 585 (Zipkin, *Proc. Soc. Exper. Biol. & Med.* **82**: 80, 1953). The data presented here describe the differences in the site of attack by caries when the two diets were fed to rats, and also the caries-potentiating effect of addition of EDTA to these diets. Four groups of thirty-six Sprague-Dawley female rats were fed either diet 580 or diet 585 (1) alone or (2) with 0.2 per cent EDTA. The results showed that 92.8 per cent of the carious lesions developed by animals on the coarse particle diet 585 were on the occlusal surfaces, while 92.0 per cent of the lesions developed by the

animals on the fine particle diet were on the smooth surfaces. With the addition of EDTA to the diets there was an increase in the number of carious lesions in the rats on both diets, the increase affected mostly the smooth surfaces. On diet 585 the average number of occlusal lesions was 2.86 without EDTA and 3.97 with EDTA, whereas the average number of smooth surface lesions was 0.22 without EDTA and 3.54 with EDTA. On diet 580 the average number of occlusal lesions was 0.50 without EDTA and 0.51 with EDTA, whereas the number of smooth surface lesions was 5.77 without EDTA and 14.57 with EDTA.

T19. A Study of the Effect of Frequency of Tooth Brushing on Oral Health. *John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The data from control clinical examinations and salivary analyses were analyzed in respect to possible relation to frequency of tooth brushing. In sixty males 21 to 28 years old, brushing once or twice per day did not appear to affect gingival hyperemia, edema, proliferation or bleeding, dental caries, lactobacilli, original pH of saliva, or pH after three hours' incubation with sugar. The amount of acid produced during incubation and salivary odor were slightly less in the twice per day group. In a group of ninety-two females brushing two, three, and four or more times per day there was a marked progressive decrease with more frequent brushing in the following: gingival hyperemia, edema and bleeding, lactobacilli, gingival debris, stains, salivary odor, and salivary debris. Salivary acid production tests showed very little difference between groups. Dental caries was importantly lower only in the group brushing four or more times per day. In a group of 155 males brushing one, two, and three times per day, frequency had little effect on the prevalence of hyperemia, edema, hyperemia in lingual gingivae, PMA of maxilla, lactobacilli, streptococci, dental caries, pH of plaque of upper ten anterior teeth, or supragingival or subgingival calculus. However, if severity of involvement were considered, more frequent brushing was associated with a decreased prevalence of severe hyperemia, edema, and PMA of maxilla and supra-gingival calculus. These results indicate that frequent brushing, even as done by the public, is highly desirable but mainly for reasons other than dental caries.

T20. Preliminary Study of the Effect of an Oral Prophylaxis on the Incidence, Signs and Symptoms of the Common Cold. *John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* As an auxiliary study to a clinical trial conducted from November, 1952, to March, 1953, weekly questionnares were distributed to subjects requesting the following information: presence or absence of a cold, date of onset, signs and symptoms. Two of the groups were given a complete oral prophylaxis at the beginning of the experiment, two others received none. The groups, number of subjects, number of colds per subject were as follows: Group I control tooth paste, 32, 1.92 colds; Group II control tooth paste oral prophylaxis, 30, 1.66 colds; Group III experimental tooth paste, 26, 1.95 colds; Group IV experimental tooth paste oral prophylaxis, 26, 1.50 colds. Analysis of the data in respect to percentage of subjects with colds without various signs and symptoms gave for Groups I to IV, respectively: nasal discharge 4, 22, 0, 15; aching 56, 74, 55, 80; sore throats 24, 30, 32, 30; cough 36, 70, 23, 55; fever 67, 86, 77, 70. Analysis on basis of percentage of colds not accompanied by various signs and symptoms gave for Groups I to IV, respectively: nasal discharge 2, 13, 0, 10; aching 29, 43, 28, 53; sore throat 13, 18, 16, 20; cough 19, 40, 12, 37; fever 33, 48, 40, 47. The data suggest that the groups receiving an oral prophylaxis had fewer colds and that the colds which occurred were accompanied by fewer signs and symptoms.

T21. Effect of Combination of Urea and Copper Chlorophyllin on Experimental Dental Caries in the Syrian Hamster. *John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* In vitro studies on salivary acid production (J. W. Hein, thesis, University of Rochester, 1952) and on salivary respiration (*J. D. Res.* **32**: 653, 1953) indicated a possible synergistic action between urea and copper chlorophyllin derivatives. The present study was designed to test this effect on animals. Twenty-seven female hamsters 25 to 28 days of age from eleven litters were distributed by littermate distribution into three groups differing as to drinking solutions as follows: Group I, 1.0 per cent urea; Group II, 0.2 per cent copper chlorophyllin; Group III, 1.0 per cent urea plus 0.2 per cent copper chlorophyllin. Keyes caries-producing diet and drinking solutions were fed ad libitum for 265 days. The resulting Keyes caries scores and standard deviations were: Group I, 9 animals, 65.1 ± 40.8; Group II, 8 animals, 62.7 ± 39.8; Group III, 10 animals, 25.1 ± 14.4. The chlorophyll derivative employed was purposely chosen because of its weak action in vitro and, in view of reports (*J. D. Res.* **32**: 705, 1953) that urea has little if any effect on dental caries in the hamster, probably had no action by itself in this experiment since the urea and chlorophyllin animals had similar scores. It appears, therefore, from the data that the conclusions obtained from the salivary studies can be extended to experimental caries in the hamster.

T22. Relation of Oral Factors to pH of Tooth Surface Following Glucose Rinse. *John W. Hein and Solvieg Schultz-Haudt, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Seventy-five male subjects, 21 to 28 years old who brushed their teeth two or three times per day, rinsed with 12.5 per cent glucose for two minutes. The pH of the ten upper anterior tooth surfaces was determined with a glass electrode approximately ten minutes after rinsing. The ten pH values were averaged and the subjects separated into three groups: Group I, eighteen subjects, pH > 6.9; Group II, forty-four subjects, pH 6.5 to 6.9; Group III, thirteen subjects, pH < 6.5. The incidence of various oral factors surveyed at the same time as the plaque tests were then determined for each group. The results expressed as means for Groups I to III, respectively, were: decayed teeth 8.0, 8.6, 8.2; DMF teeth 16.6, 20.2, 18.0; lactobacilli per milliliter saliva in thousands 151, 332, 167; streptococci per milliliter saliva in millions 56, 70, 98; teeth with supragingival calculus 0.7, 1.0, 1.0; subgingival calculus 5.6, 7.4, 10.1; PMA index of maxillary gingivae 9.4, 10.3, 16.1; PMA index (degrees 2 and 3) maxillary gingivae 1.3, 2.0, 5.9; gingival hyperemia (papillae and marginal units) severe 2.1, 3.1, 7.6; gingival edema 13.8, 18.5, 25.3; and gingival edema severe 0.9, 2.8, 5.6. Average pH for subjects with 0 to 4 cavities was 6.56, for 5 to 9 cavities, 6.88, for 10 to 18 cavities 6.70, for 19+ cavities 6.66. From this work it would appear that fall of pH of tooth surface of brushed teeth after a glucose rinse relates more to gingival disease, calculus, and streptococci than to lactobacilli and dental caries.

T23. Distribution of 1 PPM Fluoride as Radioactively Tagged NaF in Soft Tissues of Adult Female Albino Rats. *John W. Hein, Frank A. Smith, Finn Brudevold, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The relative distribution of fluoride from 1 ppm water solutions was studied by administering the fluoride with its isotope and counting NaOH digests of the soft tissues. The sample from one bombardment was given to one animal. Rat I, 170 grams, received 4.0 ml. by stomach tube and was sacrificed after ten minutes. Rat II, 147 grams, received a 1.0 ml. sample intravenously and was sacrificed after thirty minutes. Rat III, 171 grams,

received a 1.0 ml. sample intravenously and was sacrificed after ninety minutes. The results expressed as thousands of counts per minute per gram of tissue corrected to same sample activity were for rats I, II, and III, respectively: brain, 1.6, 0.7, 0.4; parotid, 11.6, 2.2, 2.0; submaxillary-sublingual, 8.6, 9.1, 0.8; thyroid, 66.4, 30.8, 13.3; heart, 7.4, 5.2, 0.5; lungs, 10.3, 6.8, 0.9; stomach, 518.8, 3.9, 0.3; small intestines, 91.4, 3.9, 2.2; large intestines, 4.4, 2.5, 0.8; liver, 10.7, 9.5, 0.9; spleen, 8.2, 6.7, 1.5; kidney, 32.8, 32.5, 17.0; adrenals, 13.3, 9.8, 2.0; uterus, 12.5, 2.1, 1.0; muscle, 9.3, 3.6, 0.5; and blood 52.6, 42.2, 16. This preliminary study indicates that the thyroid selectively concentrates fluoride from dilute solutions more than any other soft tissue studied.

T24. Further Observations on Oral, Salivary and Fecal pH in the Syrian Hamster. *Erling Johansen, School of Medicine and Dentistry, University of Rochester, and Finn Brudevold, Eastman Dental Dispensary, Rochester, N. Y.* The micro-antimony electrode has been used to study the change in pH in the oral cavity of Syrian hamsters after introduction of small quantities of sucrose in various forms. On intact tooth surfaces no consistant change was observed. In ten carious lesions with excess saliva removed before application, nine showed a drop in pH within seven minutes. The change ranged from 0.5 to 3.0 pH units with a range in readings from pH 8.2 to 5.2. To check the salivary pH readings previously reported for the hamster (*J. D. Res.* **32**: 702, 1953) and to assess the reliability of the micro-antimony electrode, further observations were made. Small samples of mixed, parotid, and sublingual saliva were collected in a capillary tube and the pH determined extraorally by depositing the sample on a specially designed glass electrode. With this technique the following readings represent averages and ranges of 20 or more samples of the following types of saliva: mixed 8.6 (8.4 to 8.9), parotid duct opening 8.7 (8.2 to 9.3), sublingual 8.7 (8.4 to 9.1). These readings were in good agreement with the previously reported values. To further check the similarity of results obtained with antimony and glass electrodes a number of readings were made by first taking the pH in certain areas in the mouth followed by removal of the saliva from these areas for determination on the extraoral glass electrode. These readings were in good agreement. Fecal pellets moistened in distilled water gave readings from 5.0 to 5.5.

T25. A Quick and Non-Injurious Method for Repeated Blood Sampling From the Oral Region of Syrian Hamsters and Rats. *Erling Johansen, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* In the method about to be described the blood is drawn from the area between the mandibular incisors. An instrument similar to the one previously described for oral examination of rodents is used for immobilization of the unanesthetized animal. By extending the openings for the mandibular incisors in the mandibular stabilizer down to the frame, the labial aspects of the mandibles become exposed and accessible. To free both hands for experimental procedures the animal is mechanically locked in the device by rotating the maxillary adjustment lever back until it rests on the animal's head or neck. The animal-instrument assembly is attached to a suitable holder mounted on a ringstand with a platform supporting the animal's back. With the animal in this position the dark outline of the blood vessel from which the blood is drawn is seen labially in the midline of the mandibular arch. To aspirate the blood into a syringe, a 26 gauge ¼ inch needle (in young animals 30 gauge) is used. The needle is inserted in the midline approximately 1 mm. below the most apical part of the free gingiva of the incisors at an angle of 45° from above downward. Most commonly a smooth, even flow of blood is obtained. Sometimes, however, air bubbles also are aspirated. This occurs most frequently when the bevel of the

needle used is too long or when the gauge of the needle is not right for the size of the animal. With this technique more than ten samples have been obtained from individual animals and samples up to 1 c.c. have been drawn with ease.

T26. Dental Caries and Gingivitis in Tel Aviv, Israel. *M. Laufer, Department of Education and Public Health Services, Municipal Corporation of Tel Aviv-Jaffa.* As part of a countrywide survey of the prevalence of dental caries, gingivitis, and other dental disorders 7,454 pupils ranging from 13 to 17 years of age of the 50,000 school children were examined in Tel Aviv in September, 1952. No other survey was made previously in this country based on DMF and PMA. Special attention was given to control divergence in observations among different examiners and fifteen classes with 433 children of 14 years of age were examined twice to recheck results. Findings were compared with those on children in Philadelphia (Cohen and Massler, 1952). The DMF in Tel Aviv was 3.06, less than one-half of that in Philadelphia and less than one-third of that in New Zealand. Tooth loss per 100 children was 108 in Philadelphia, 41 in New Zealand, and only 9 in Tel Aviv. Tooth loss among immigrants was higher than in native children. PMA index of Tel Aviv children was 0.80, about one-sixth of the 4.9 reported for Philadelphia children (Massler, Cohen, and Schour). Incidence of malocclusions was lower in Tel Aviv children than those in U. S. A. (Moore, 1948). Enamel hypoplasia was much less than generally reported (Sarnat and Schour, 1941). Fluorine content of Tel Aviv drinking water varies from 0.3 to 1.2 ppm in thirty-five different wells piped in separately and not pooled. There is relatively low sugar consumption in Tel Aviv; about one-fourth of U. S. A. sugar consumption. The low PMA value in Tel Aviv cannot be ascribed to superior oral hygiene but possibly to a more detergent action and abrasive character of the diet. Public dental health service has been proved to influence reduction of tooth loss. Despite higher DMF value in New Zealand (10.01) than in Philadelphia (8.1), tooth loss is lower in New Zealand. In Tel Aviv tooth loss is even lower than in New Zealand (0.09 against 0.41). Two and nine-tenths per cent of teeth are lost in Tel Aviv, 4.1 per cent in New Zealand, and 13.3 per cent in Philadelphia. Filling rate proved to be highest in New Zealand—86 per cent of teeth attacked, Tel Aviv 68.7 per cent, Philadelphia 42 per cent. The explanation is that in New Zealand one dental operator is responsible for 500 pupils, in Tel Aviv one dentist for 3,000 pupils (part time). Of special interest was the extraordinarily low DMF rate among ninety-four Yemenite children recently immigrated (0.58). Their PMA index (1.6) was twice as high as in Tel Aviv (0.8). While there may be a genetic factor causing the lower DMF value in Yemenite children, the reason for the higher PMA rate may be the absolute absence of oral hygiene.

T27. In Vivo Absorption of Fluorides From the Gastrointestinal Tract. *R. C. Likins and I. Zipkin, National Institute of Dental Research, Bethesda, Md.* Rats weighing 110 to 120 grams were starved for seventy-two hours and various fluorine compounds were given by stomach tube, at a level of 200 μg F. Thirty minutes later the rats were sacrificed, the entire gastrointestinal tract was excised, analyzed for fluorine and the per cent of administered F absorbed was determined: NaF, 49.6 per cent; Na_2SiF_6, 40.9 per cent; Na_2PO_3F, 42.2 per cent; KPF_6, 77.2 per cent; and $(C_2H_5)_4NPF_6$, 67.7 per cent. Thus NaF, Na_2SiF_6, and Na_2PO_3F are absorbed at similar rates, whereas KPF_6 and $(C_2H_5)_4NPF_6$ are absorbed at faster rates. The absorption of other variously bonded fluorine compounds is now being investigated.

T28. The Effect of Prior Fluorine Exposure on the Deposition of Radiocalcium in the Rat. *R. C. Likins, I. Zipkin, A. C. Steere, and F. J.*

McClure, National Institute for Dental Research, Bethesda, Md. Female rats were given either sodium chloride (20.50 mg. total Cl) or sodium fluoride (2.05, 4.10, or 20.50 mg. total F) by stomach tube during the sixty days following weaning. Next, all were injected intraperitoneally with 2 μc of carrier-free $Ca^{45}Cl_2$ on each of the five succeeding days. Twenty-four hours after the final injection, the animals were sacrificed and autoclaved. The femurs and mandibles were removed and analyzed for fluorine and radiocalcium. The mean percentages of fluorine in femur and mandible ash, respectively, are: for control, 0.0068 and 0.0067; for experimental, in ascending order of fluorine intake, 0.0489 and 0.0431; 0.0950 and 0.0826; 0.4064 and 0.3950. Corresponding mean percentages of Ca^{45} retained per milligram of ash are: for control, 0.0344 and 0.0354; for experimental, 0.0311 and 0.0325; 0.0313 and 0.0321; 0.0335 and 0.0330. The data indicate that previous exposure to fluorine did not significantly alter the deposition of labeled calcium in these bones.

T29. Effects of Sterilizing Agents on the Growing Dentin in the Incisor of the Albino Rat. *Maury Massler and J. G. Perreault, College of Dentistry, University of Illinois, Chicago, Ill.* Cavities were prepared in incisors of albino rats and a variety of sterilizing agents applied under standard conditions for one minute, ten minutes, and two days. The cavities were then filled with a zinc oxide-starch-water mixture covered with wax and the animals were sacrificed two and seven days later. Effects of the drugs on the pulp and odontoblasts were reflected in alterations in the calcification and formation of the developing dentin, which is a delicate indicator of alterated physiology of these cells. Alterations in dentin calcification occurred long before changes in cell morphology were observed. It was found that the use of eugenol produced minimal changes in the calcification of the growing dentin and no changes in the pulp and odontoblasts, while silver nitrate caused death of odontoblasts and complete arrest in dentin formation. The order of increasing effects on the dentin and pulp was: eugenol, Cresatin, Zephiran, beechwood creosote, eucalyptol, phenol, alcohol 95 per cent, heat, saturated solution of silver nitrate, and ammoniacal silver nitrate. Silver nitrate reduced by formaldehyde solution was more injurious than when reduced by eugenol. With the exception of eugenol, all effects were greatly exaggerated when these drugs were sealed in the cavity preparation for two days.

T30. Effects of Filling Materials on Growing Dentin in the Incisor of the Albino Rat. *Maury Massler and Maria Silberkweit, College of Dentistry, University of Illinois, Chicago, Ill.* Cavities were prepared in incisors of albino rats and filled with a variety of filling materials to determine the effects of these materials on the young pulp and odontoblasts. The effects were reflected in alterations in the calcification and formation of the dentin and occurred before morphologic changes could be observed in the odontoblasts and pulp. Alterations in dentin calcification are delicate indicators of the altered physiology and function of the odontoblasts. Animals were sacrificed two and seven days after operation. It was found that the use of zinc-oxide-eugenol cement produced minimal alterations in the calcification of the developing dentin and no morphologic changes in the pulp or odontoblasts, while silicate cements and copper cements produced death of odontoblasts and marked arrest in dentin formation and calcification. The order of effects increased from zinc-oxide-eugenol to amalgam, self-curing acrylics, zinc oxyphosphate cements, silicates, and copper cements. On the basis of this study, the use of zinc-oxyphosphate cements as a protective base in deep dentinal cavities in children is questioned. Cavity varnish was found to be completely ineffective in protecting the pulp against injury by silicates. Calcium compound liners were found to be very effective in this respect.

T31. The Pulpal Response of the Rat Molar to Cavity Preparation and Sodium Fluoride. *Charles G. Maurice and Isaac Schour, Departments of Dental Therapeutics and Histology, University of Illinois, College of Dentistry, Chicago, Ill.* The effect of cavity preparation and filling with "aquadont" alone (zinc oxide two parts, zinc sulfate one part, and corn starch one part) and "aquadont" plus 1, 2, 4, and 30 per cent by weight of sodium fluoride or with "aquadont" preceded by flooding with a 4 per cent aqueous solution of sodium fluoride for five minutes was studied histologically in the upper right first maxillary molar of fifty-four male albino rats 60 to 100 days of age. Three animals in each group were sacrificed one, six, and 32 days following the preparation and filling of the cavity. The histologic findings were confined to the area subtending the cavity along the course of the cut dentinal tubules. The findings in the teeth filled with 1, 2, 4 per cent NaF or flooded with 4 per cent NaF for five minutes were similar to those in teeth filled with "aquadont" alone. These showed in the twenty-four-hour period disturbed predentin, an increase in the number of cells, a loss in the distinctness of the odontoblastic layer, replacement of some odontoblasts by a preodontoblastic type of cell, and numerous and dilated capillaries. The six-day specimens showed partial recovery from the initial injury with a predominance of young odontoblasts. The thirty-two-day specimens showed recovery which was essentially complete with a reparative process consisting of a deposit of secondary dentin. In the teeth filled with aquadont plus 30 per cent NaF, alterations were observed which were similar in type but more severe in degree than in the previous groups. In the six-day specimens there was either very slight or no recovery. The thirty-two-day group showed incomplete recovery and a greater deposition of secondary dentin than in the previous groups.

T32. Method of Obtaining Torque Using Round Wire in the Edgewise Technique. *Henry I. Nahoum, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* The object of this study is to present a simple technique for the edgewise mechanism that can produce the three orders of forces on teeth. Since Gottlieb pointed out that a rectangular edgewise arch is capable of setting up pathosis and insidious forces that counteract each other during mass movement of teeth, various clinicians have attempted to design appliances that could separate the forces and eliminate undesirable movements. This method utilizes round resilient arch wires from .016 to .022 inch in diameter. The wire is contoured to the desired arch form, using full or sectional arch. Torquing or stabilizing bends are made by crimping the wire at exact point that wire will sit in the edgewise bracket when the arch is ligated in place. The crimp endows round wire with properties of rectangular wire. Torque is effected by holding plier in such a way that it will create a crimp in exact direction that tooth is to be torqued. With this technique operator can resolve various forces within one arch wire. Although this or other techniques can perform first two orders of movements readily, chief merit of this method is that torque force can be used with confidence and definite direction in a simple manner with selection of anchorage. No compensating bends are required as with rectangular arch wire. Force used is gentle and acts over a long distance. Torque incorporated in an arch in this manner is "visible" and can be used to demonstrate applications of this force in orthodontics. The following results have been obtained: 1. Rapid and gentle movement of canines in extraction cases with sectional arches using a safety-pin-type spring vertical loop. 2. Torquing of a single tooth. 3. Torquing of two adjacent teeth in different directions simultaneously. 4. Torquing of individual segments, anterior or buccal. With this technique, torque which has been considered an insidious and elusive force can be understood and easily controlled.

T33. Some Experimental Observations on the Diet and Oral Hygiene in Relation to Dental Caries. *R. B. Nevin, Department of Basic Dental Sciences, Otago University, Dunedin, New Zealand.* The effects of cookie/saliva mixture on the rate of diffusion of lactic acid from between closely spaced glass rods support the conclusion that changes in the physical properties of food may have a greater effect on caries incidence than variations in sugar content. Tooth brushing experiments conducted similarly show that only when there is no barrier to free interchange of fluids can the toothbrush effectively clear narrow spaces of sugar and acids. These experiments and additional experiments with a lower partial denture equipped with removable crowns of natural teeth in correct approximal relation show that sticky foodstuffs are not easily dislodged from narrow spaces even by very vigorous brushing. Insufficient or inefficient brushing would tend to force such material further into the space. Impacted or adherent debris may result in prolonged retention of sugar and acids in narrow spaces, even when some toothbrushing is undertaken. Spacing of teeth is an important factor in food retention: slight spacing may have a greater locking effect than tight contact. Beyond a certain critical value of spacing, clearance of debris becomes satisfactorily efficient. Further evaluation of toothbrush techniques and their effectiveness is necessary with special reference to different age groups. Much more research on the problem is required. Suggested intra-oral methods include dentures with removable and adjustable crowns of natural teeth and the use of two-part inlays in which the interproximal contact area forms a wedged slide, movable mesiodistally in the main cemented part, and locked by a screw or small wedge.

T34. The Diet and Mastication; Their Effects on Diffusion and the Inception of Dental Caries. *R. B. Nevin, Department of Basic Dental Sciences, Otago University, Dunedin, New Zealand.* Measurements were made by a platinum electrode of the diffusion rates of lactic and hydrochloric acid from between glass rods spaced 0.08 mm. and 0.15 mm., immersed in buffer solutions and saliva. Movement of glass slides and of closely adapted areas of impression compound simulated masticatory effects. Obstruction of the interrod space by measured amounts of filter paper simulated the presence of food debris. The experiments indicate that the properties of food and food/saliva mixtures should be evaluated in terms of permeability, adhesiveness, buffer capacity, acidity, and "packing quality," a combination of plasticity and particle size (masticatory function important). Sugar content and sugar-forming potentialities are also important, but changes in physical properties may be more important than variations in sugar content. Sugar solutions are much less dangerous than sugar incorporated in sticky foods. High sugar concentrations are not necessary to explain either the inception of caries or the shape of the lesion; concentration gradients in narrow spaces are explanatory. In fissures and interproximal areas, caries may be a relatively continuous but fluctuating process; exposed surface caries is more intermittent and requires greater sugar concentrations. Local variations in caries incidence are largely controlled by the amount of food debris retained. This is determined by the shape, size, and position of the teeth and the degree of muscular activity. The buffer constituents of saliva can be as effective as chemical buffers under some circumstances where it is unlikely that the whole saliva can penetrate.

T35. Tooth Physiology: Permeability. *P. Pincus, Dental School, University of Melbourne, Australia.* Leicester (Biochemistry of Teeth) stated that while an erupted tooth is capable of physiologic changes, it is uncertain whether enzymes are involved. A method of demonstrating such changes is described, permitting investigation of enzyme action. Chemical agents normally present

in the body are shown to pass from odontoblasts through dentin and enamel to be demonstrated free in fluid surrounding the enamel. Recently (*Aust. J. Dent.* **56**: 331, 1952), physiology of living odontoblasts has been studied within a few minutes of extraction of the tooth. A tooth crown complete with odontoblasts but otherwise free of pulp forms a "cup." Into such a cup (kept at 37° C.) P_{32} is placed in an isotonic nutrient medium (Na glycerophosphate in glucose/Pannett Compton solution saturated with O_2). Passage of P_{32} through the tooth by way of the odontoblasts, dentin, and enamel occurs. The odontoblastic layer is found readily capable of hydrolyzing glycerophosphate, releasing phosphate ions, though these are absent from the original solution. Further tests on living odontoblasts are being made. The method offers advantages over tests on animals; human teeth are used. For choice, upper incisor or canine teeth (caries-free) from young subjects are used though these are scarce. Sound premolars removed for orthodontic reasons have also been used.

T36. The Effect of 625 r, 2,000 KVP, Total-Body X-Radiation Upon the Salivary Glands in Swiss Mice. *Jens J. Pindborg, Royal Dental College, Copenhagen, Denmark, James A. English, Naval Medical Research Institute, Bethesda, Md., and Paul E. Boyle, University of Pennsylvania, Philadelphia, Pa.* One thousand healthy white Swiss mice (982 male, 8 female), weighing 15 to 18 grams each were exposed in groups of 200 to the horizontal beam of a 2,000 KVP G-E industrial x-ray machine. The animals received 625 r single dose delivered at the rate of 14.5 r.p.m. at a target-skin distance of 2 meters, Ma. 3, no added filter. The progression of general morphologic lesions on these same mice have been reported by B. G. Lamson and J. L. Tullis (*Mil. Surgeon* **109**: 281, 1951). The mice were sacrificed serially, ten mice each hour for the succeeding ninety-nine hours. Ten mice died and were excluded from the study. Ten unirradiated mice were used as controls. Histologic sections were prepared from the parotid, submaxillary, and sublingual salivary glands after fixation in Zenker-formol solution, and sections were stained with hematoxylin and eosin dyes. A careful study was made of these tissues. No changes were found in any of the salivary glands from these mice sacrificed during the four-day (plus) period which could be ascribed with certainty to radiation damage. Changes of the following kinds were randomly and infrequently observed: alteration in nuclear size in submaxillary gland acini, vacuolation of alveolar cells in each kind of gland, shrinkage in size of acini or tubules, with reduction in number of cells. None of these changes were sufficiently regular to be considered significant.

T37. Study of Dental Caries in Children 1 to 6 Years of Age as Related to Socio-economic Level, Food Habits and Oral Hygiene. *Bhim Sen Savara and Theodore Suher, University of Oregon Dental School, Portland, Ore.* These findings are supplementary to an earlier report presented in 1953. Trained interviewers obtained home interviews from the parents of 279 children and the data were analyzed using correlation technics. No association was found between dental caries incidence (def teeth) and (a) desserts consumed at lunch or at dinner, (b) economic level of the parents as estimated by the interviewer, (c) type of father's employment (unemployed, unskilled, skilled, white collar, managerial or owner, and professional), and (d) cultural level of the parents (estimated by the number of subscribed magazines). Absence of association was found between income of parents and number of times per week the child consumed (a) ice cream, cake, pie, gelatin, cookies, and candies, (b) fresh fruits, and (c) canned fruits. No significant association was found between age of the child and number of

times per week the child ate (a) cake, pie, ice cream, gelatin, cookies, and candies, (b) canned fruits, and (c) fresh fruits. Frequency of toothbrushing and whether the child brushed his teeth at rising, before breakfast, after breakfast, after lunch, after dinner, or before retiring had no relationship to the age of the child, incidence of dental caries, or the income of the parents.

T38. The Sodium and Potassium Concentration of "Resting" Saliva From Individual Gland Pairs in Man. *Leon H. Schneyer and Ford Turner, School of Dentistry and Medical College of Alabama, University of Alabama, Birmingham, Ala.* The separate collection of submaxillary and sublingual salivas has been made possible by modification of the "segregator" of Pickerill (Schneyer, 1954). The use of this device together with the parotid cup provides opportunity for the investigation and comparison of component substances of the salivas of the three pairs of major salivary glands in man. This report is concerned with an analysis of the sodium and potassium concentrations of salivas from the individual gland pairs. The salivas used were "resting" secretions in the sense defined in another report (Schneyer and Levin, 1954). Sodium and potassium concentrations were determined with the use of a Beckman spectrophotometer and flame attachment. Fourteen males in good health whose ages ranged from 20 to 30 years were used as subjects. The arithmetic mean of the sodium concentration of the total mixed salivary secretion was 7 meq. per liter. The mean sodium concentration of the submaxillary salivas was 4 meq. per liter. The mean sublingual sodium concentration was 15.0 meq. per liter, while the mean sodium concentration of the parotid secretion was 6 meq. per liter. In the case of potassium, the mean values were 17 meq. per liter for the total mixed saliva, 19 meq. per liter for the submaxillary saliva, 19 meq. per liter for the sublingual saliva, and 20.0 meq. per liter for the parotid saliva. The degree of variation in the sodium values was much greater for the salivas from all three pairs of glands, separately and as a total secretion, than was the case for potassium. Moreover, the potassium concentration appears to be about the same in the secretion from each type of gland, while in the case of sodium, the concentration appears to be greatest in the sublingual and lowest in the submaxillary.

T39. Rate of Flow of "Resting" Saliva From Individual Gland Pairs in Man. *Leon H. Schneyer and Lawrence K. Levin, School of Dentistry and Medical College of Alabama, University of Alabama, Birmingham, Ala.* Although data are available for the rate of flow milliliters per minute of the "resting" parotid, mandibular (mixed submaxillary and sublingual), and total mixed salivas, measurements of the volume flow of the separate submaxillary and sublingual secretions have not been made. The modification of the Pickerill segregator recently described (Schneyer, 1954) now permits collection of relatively pure submaxillary and sublingual secretions. Consequently, measurements of volume flow from each pair of major salivary glands have now been made. The salivas were collected under conditions of minimal sensory stimulation. Within the limits imposed by the method of collection (the Lashley cup for the parotid, the segregator for the submaxillary and sublingual, and the saliva ejector for the total mixed saliva), the secretions may be considered "resting." Fifteen healthy men from 20 to 30 years of age were used as subjects. The arithmetic mean of the volume flow for the total mixed secretion was 0.70 ml. per minute. The mean submaxillary volume flow was 0.29 ml. per minute, while the mean sublingual flow was 0.016 ml. per minute. The mean parotid flow was 0.19 ml. per minute. Thus the submaxillaries account for the bulk of the "resting" saliva (41 per cent); the parotids appear to produce about 26 per cent and the sublinguals only 2 per cent of the total "resting" saliva. Of the total flow, 31 per

cent is unaccounted for after addition of the volume flow values from the large pair glands. This may be considered to be at least a preliminary approximation of the extent of the buccal secretion in man.

T40. The in vivo Fermentability of Sugars and Polyhydric Alcohols in Carious Lesions. *Donald E. Shay, University of Maryland, Dental School, Baltimore, Md.* An investigation was conducted to compare the in vivo fermentability (acid production) of sucrose, dextrose, glycerin, and sorbital solutions in carious lesions. After rinsing for two minutes with test solutions, in 2 per cent concentrations, pH readings were made at intervals of two, ten, and twenty minutes. The pH readings were made with an antimony electrode on the saliva and 225 carious lesions in 33 clinical patients. Averages of the in vivo pH determinations and the statistical analyses for significance at the 0.05 level show that rinsing with either glycerin or the two sorbitol solutions does not permit the production of enough acids to cause the pH to drop below the critical decalcification level for enamel (5.5). After rinsing with a solution of either dextrose or sucrose, average pH values below 5.5 were recorded at the two- and ten-minute intervals which rose but did not revert to the control levels after twenty minutes.

T41. The Effect of Complete Mouth Rehabilitation on the Salivary Lactobacillus Count. *I. L. Shklair, H. R. Englander, and L. M. Stein, Great Lakes Naval Training Station, Great Lakes, Ill.* This is a study to show the effect of complete mouth rehabilitation on salivary lactobacillus counts. A group of forty-seven naval enlistees from 17 to 21 years of age were segregated for the express purpose of restoring all carious teeth and removing those that were unrestorable. These persons presented a clinical and radiographic history of rampant decay, and only cursory evidence of previous dental treatment. Two fasting saliva samples were analyzed prior to the initiation of clinical program, and subsequent samples, similarly taken, were analyzed at bimonthly periods for four months following completion of the operative and surgical procedures. Eighty per cent of the cases showed an initial drop to 500 colonies or less of lactobacilli per milliliter and many became negative. The remaining cases, with one exception, exhibited a reduction of 65 to 98 per cent over the preclinical counts. Most cases remained at this reduced level for two to three months, and then began to rise. The level of rise did not approximate the preoperative counts, and a few cases remained negative throughout the study. The period of observation was limited to the recruit training period of four months.

T42. Parasitic Fauna of the Syrian Hamster. *Wayne W. Wantland, Division of Science, Illinois Wesleyan University, Bloomington, Ill.* Examination of 412 Syrian hamsters revealed the following animal parasites, their location and incidence: 1. Trichomonad flagellates in intestine, particularly the caecum (99.27+ per cent). Animals in which trichomonads were observed harbored *Tritrichomonas muris, Tritrichomonas wenyoni, Tritrichomonas minuta* and a form resembling *Trichomonas microti.* Another trichomonad, unlike any reported in the literature, is described. 2. *Endamoeba muris* in the caecum (33.0 per cent). 3. *Giardia* resembling *Giardia muris* in the small intestine (12.37+ per cent). 4. *Chilomastix bettencourti* in the caecum (9.22+ per cent). 5. *Hymenolepis nana* in the small intestine (0.72+ per cent). 6. *Cysticercus fasciolaris* in the liver (0.24+ per cent). 7. *Syphacia obvelata* in the caecum (0.48+ per cent). 8. *Musca domestica* larvae in the lower colon and rectum (0.24+ per cent). Hamsters from eight sources were examined. No significant differences with regard to parasitism of male and female hamsters were noted. Older hamsters (2 years and over) usually showed smaller numbers of intestinal protozoa than did younger animals. Evidence indicates that hamsters become

infected with the various intestinal protozoa shortly after weaning (18 to 24 days) by ingesting feces containing the parasites. The parasites observed are described with reference to morphology, life cycles, and activity in laboratory preparations. Results from this work indicate that effects from parasitism in the hamster should be taken into account in dental caries, cancer, and nutritional studies employing this animal. It is suggested that precautionary measures against transmission of infections from animal to animal and from animal to human being should be carefully observed by research workers.

T43. The Three-Dimensional Structure of the Enamel Rod. *M. L. Watson and J. K. Avery, School of Medicine and Dentistry, Departments of Radiation Biology and Dental Research, University of Rochester, Rochester, N. Y.* Thin sections of the developing enamel of the hamster lower incisor were cut at various angles to the enamel surface and the tooth axis and examined in the light and electron microscopes. From these observations a tentative reconstruction of the three-dimensional form of the enamel rod has been made. In the young enamel of this tooth the enamel rod consists of a hexagonal tube about 6 μ in diameter and an intratubular material. The tube is divided by parallel partitions inclined to the axis at an angle of roughly 45°. The partitions terminate at opposite vertices of the hexagonal tube. The walls and partitions are at uniform composition throughout in the sense that there is no discernible "skeleton" or rod sheath such as might arise from the ameloblast cell wall. These structures consist of long, parallel-lying, ribbon-shaped crystals plus presumably an organic component which is not visible in the undecalcified sections. In the walls the crystals lie roughly parallel to the axis of the rod and in the partitions they run parallel to the long dimension of the partition. The tube is filled with material which is indistinguishable from the wall and partition material except for the crystal direction which is different. The intratubular fibers run approximately at right angles to both the wall and partition fibers. The walls and partitions are laid down together, but the intratubular material is deposited later.

T44. Developing Dentin as Seen in the Electron Microscope. *M. L. Watson and J. K. Avery, School of Medicine and Dentistry, Departments of Radiation Biology and Dental Research, University of Rochester, Rochester, N. Y.* Section of the dentin of the hamster lower incisor were prepared and studied with the electron microscope and the following observations were made: Between the border of the predentin and the odontoblasts there is seen in section a strongly osmiophyllic broken line. This line is the profile of a surface (the "predentinal boundary") about 0.1 μ thick. There is an abrupt change in the appearance of the cytoplasm of the odontoblasts on one side of the predentinal boundary and of the contents of the predentinal tubules on the other side. Dentin collagen appears to be an extracellular product. In uncalcified dentin the collagen fibers range in size from approximately 100Å to 600Å in diameter, the smallest of these lying near the surface of the predentin tubule. There is a sharp demarcation between the calcified and uncalcified dentin which we term the "calcification front." Near this zone the collagen fibers have a diameter of 300Å to 600Å. Initial mineralization at the calcification front is highly localized at individual collagen fibers. Within 0.5 μ of the calcification front, calcification has spread into the extrafibrillar spaces.

T45. Reparative (Secondary) Dentin in the Rat Molar Following Cavity Preparation and Fillings. *Stanley Weider, Isaac Schour and Clive Mohammed, Department of Histology, University of Illinois College of Dentistry Chicago 12, Ill.* The effects of cavity preparation and filling with zinc oxide and eugenol, "aquadont," and a 50 per cent mixture of "aquadont" and tri-

calcium phosphate, as well as unfilled cavities, were studied histologically in the upper right first molar of seventy rats approximately 60 days old. These animals were sacrificed two and three weeks after the operation. In all specimens, the pulp responded with the formation of reparative (secondary) dentin. At twenty-one days there was a strong relation between cavity depth and amount of reparative dentin ($r = 0.75$). The average amount of reparative dentin in cavities 300 to 400 μ from the pulp was 45 to 75 μ. Zinc oxide and eugenol and cavities left open to saliva delayed the initiation of reparative dentin formation. At twenty-one days, however, the amount formed was equivalent to that of the other series.

T46. Studies of Ovarian Function in Periodontosis, II. *Roslyn Wiener, Maxwell Karshan and Benjamin Tenenbaum, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Thirty-three cases of periodontosis (including twenty cases of a previous study reported, *J. D. Res.* **30**: 460, 1951) were studied in an attempt to evaluate the influence of ovarian function on this disease process. Indices used were vaginal smears and basal body temperature during a complete cycle. In sixteen of these cases simultaneous smears were taken from the left maxillary buccal mucosa and studied from two points of view: (1) the possible existence of a distinctive oral smear picture in periodontosis, and (2) correspondence with the vaginal smear in demonstrating the quality and periodicity of ovarian activity. Of the thirty-three patients studied, nineteen showed a good ovarian cycle, eight were fair, three were poor and three were anovulatory, demonstrating a wide range of ovarian activity in the group. Studies of parallel vaginal and oral smears in sixteen patients showed in eleven subjects good correspondence between the two series for all degrees of ovarian function including anovulatory. Three of these eleven patients showed essential cyclic correspondence, but indication of ovulation two to three days later in the oral series as compared with the vaginal smears and basal body temperature records. In 5 patients, there was no correspondence between vaginal and oral smears, the latter failing to show a distinctive cyclic pattern. There was no characteristic oral smear picture of periodontosis. The hormonally induced changes throughout the ovarian cycle were shown in the oral as well as the vaginal smears in eleven of the sixteen patients despite varying degrees of periodontal disease.

T47. Dental Caries Studies in Israel. *Max M. Weinreb and David Ben-Shushan, H.Q. Surgeon General, Israel Defence Army.* The caries experience of a group of 7,727 young servicemen 18 to 30 years of age was assessed by simple clinical examination of the teeth and scoring the DMF rate for the group. The caries rate was also computed separately in the upper and lower arches in order to establish the relative susceptibility of each. The caries experience of the first permanent molars was also calculated in order to test the validity of using the first permanent molar as an index of the total caries experience at this age. The number of DMF teeth was 3.66 per person at the age of 18 years. From 19 to 30 years of age this figure rose steadily and reached 8.62 at the age of 30. These numbers are rather low when compared with statistics in other countries. More teeth were affected in the upper jaw than in the lower jaw. The rate varied from 2.10 at 18 years to 5.26 at 30 years for the upper teeth, and from 1.55 to 3.36 for the lower teeth at the same ages. The lower first permanent molars, however, were more affected by caries than were the upper ones. (Upper molars: 0.87 at 18 years; 1.37 at 30 years. Lower molars: 1.10 at 18 years, 1.64 at 30 years of age.) The coefficient of correlation for the number of DMF teeth and DMF first molars was +0.317 and thus showed that there was no correlation between caries prevalence in

the first permanent molars, when compared to the total caries experience. Therefore the first permanent molar cannot be used as an index for the total caries experience at the ages of 18 to 30 years.

VI. Executive Proceedings

A. Council Activities

Activities Between General Meetings

In the recess following the 1953 general meeting, the Council approved the details of the 1953-1954 budget for the operation of the Secretary-Treasurer's offices and nominated the candidates for the general offices listed on the official ballot as Council candidates. A Council committee (Kesel, Williams, Sognnaes) studied the credentials of all nominations to membership received during the year.

Council Meetings, March 19 and 21

Council members in attendance at one or both sessions: Allen, Armstrong, Arnold, Boyle, Burrill, Dean, Grainger, Grossman, Hall, Hatton, Hill, Hodge, Jay, Kitchin, Krasnow, Kreshover, Manly, Orland, Paffenbarger, Robinson, Scott, Swanson, and Volker.

The Program Committee was authorized to increase its membership for the current year by the addition of one or more assistants. A dues assessment for the 1955 calendar year of $10.00 for each dues-paying member was authorized, out of which $7.00 was appropriated for subscriptions to the Journal of Dental Research and $3.00 for the general fund. After long discussion a motion to remit the dues of members in countries outside of the United States and Canada for the 1955 year was lost. On motion of the officers of the Association were advised to investigate the problems associated with the collection of dues and subscriptions to the Journal of Dental Research from members in countries outside of the United States for the 1955 calendar year and to proceed in accordance with their findings up to the time of the 1955 general meeting. Dr. Paffenbarger's motion which follows was seconded and carried: The Council should authorize the Board of Trustees to explore the necessary steps to incorporate the International Association for Dental Research and that the Board report to the Council at the 1955 annual meeting on the cost of the necessary procedures to effect incorporation.

A communication was read from the St. Louis section with reference to advertising in the Journal of Dental Research and later at the request of the St. Louis Council member was withdrawn. A list of names of those whose nominations were found to meet all the requirements of the Association for membership according to the report of the Committee was read and was ordered for submission to the Association as approved by the Council (p. 726). A tentative budget for the 1955 calendar year for the Secretary-Treasurer's offices was approved and disbursements authorized as follows: total, $1,700; gratuities, $500.00; clerical, $200.00; postage, $150.00; supplies, $100.00; printing, $500.00; travel and miscellaneous, $50.00; and Novice Award, $100.00.

B. Executive Sessions

First Session, Friday Morning, March 19, 1954

The meeting was opened by President Arnold with brief, appropriate remarks. The minutes of the 1933 (Thirty-first) general meeting were approved as printed in the Journal of Dental Research, pages 628-723, Volume 32 after the following corrections had been made: on page 721 in the list of new members correction of the spelling of the names of Gerhard M. Brauer and H. I. Nahoun, and the insertion of the name of Robert I. Gottsegen of New York. B. O. A. Thomas and Ned Williams were appointed as tellers to receive and poll the ballots cast for the officers for the 1954-1955 Association year. The Secretary-Treasurer reported on the status of the Association as follows;

As of the first of this year this Association had nearly 800 members, of which more than three-fourths are from North America; and next year it will be 35 years old. It still operates with the same machinery as furnished by its founders, at least with little

essential change, and is designed primarily to administer its affairs during its growth and in a social and economic period that seems very remote now. Its officers were wholly volunteers; they either did the work with their own hands or imposed it on the clerical staffs of the institutions by whom they were employed. Dues were nominal, and if or when collected were not spent. A journal was published but members of the Association subscribed to it voluntarily, but as a rule not many volunteered. The greatest change has taken place in the conduct of the JOURNAL OF DENTAL RESEARCH, but wholly within the framework of the constitution. Dues now include a subscription to the Journal and the constitutional limit of dues has been raised from $5.00 to $10.00.

The Association now has at least ten times as many members as it did at the end of its fifth year of existence. Scientific sessions of the first five years, if and when scheduled, were limited to a single half-day session which was more than adequate to present all the titles offered for presentation. Now twelve half-day sessions are not enough. A large share of the pages of the first volumes of the JOURNAL OF DENTAL RESEARCH were devoted to articles that were not the presentation of research. Now all active members of the North American sections subscribe to the JOURNAL and two of its officers receive considerable but inadequate gratuities. Dues are relatively small and are used as far as they reach to support the activities of the Association. There is a considerable number of members in countries outside of North America but nothing much is being accomplished to realize the many advantages that should accrue to both parties in such an arrangement. In the course of the years the Association has created a number of sections that have not realized the promises seemingly offered for growth and development at the time of their formation. These and other problems offer a challenge to the Association and demand more than just routine attention, perhaps even an overhauling of the constitution and by-laws. The group unit called a division in the constitution (including all members in a single geographic entity) seems to be the key to the administrative procedures to be applied to the operation of the Association at the international level. In spite of this the constitutional treatment of the division is not only limited, it is almost lacking, and confined to little more than one sentence.

During the coming year, it would seem not only profitable but also absolutely essential that this subject should be given thorough study and that the 1955 general meeting be supplied with one or more reports ready to be adopted as amendments to the constitution and by-laws. Two committees are suggested: one on administration in general and one on the international situation and the operation of divisions.

Much of the work of administration falls into nearly independent projects, such as: (1) Collection of dues and the maintenance of membership files. (2) Assemblying the program for the annual meeting and the conduct of its proceedings. (3) The collection and evaluation of the nominations to membership and the presentation of the findings to the Council. (4) Secretarial services to the Council. (5) Routine correspondence and the preparation of bulletins and memoranda to members. (6) The collection of manuscripts and the processing of the same for publication in the JOURNAL and such other subjects that have to do with the JOURNAL OF DENTAL RESEARCH. For the coming association year I propose to operate these as independent projects, that is as far as is consistent, and assign them as follows: 1 and 5 to the Assistant Secretary-Treasurer; 2 to the Program Committee; 3 to the Committee on nominations to membership; 4 to the Secretary-Treasurer; and 6, of course, to the editor.

C. COMBINED FINANCIAL REPORT OF THE SECRETARY-TREASURES (CHICAGO AND LOUISVILLE) FOR THE 1953 CALENDAR YEAR

Receipts

General Fund (dues)	$ 832.00	
JDR Fund (subscriptions from members)	3,610.00	
C. V. Mosby Co. (gratuities)	1,800.00	
Interest on endowment	32.50	
Total		$6,274.50

Disbursements

Postage	$ 121.46	
Clerical	161.86	
Printing	479.33	
(overdrawn because of inclusion of reprints of the biographical directory of membership)		
Supplies	66.18	
Telephone, telegraph, and express	7.16	
Meeting expenses, travel, and miscellaneous	505.79	
Refunds	43.00	
C. V. Mosby Co. (block subscription to JDR)	3,773.00	
Gratuities to the Publication Committee	1,500.00	
Novice Award	100.00	
Total		$6,762.78
Gross deficit		488.28
After deduction of refunds and Novice Award, net deficit		345.28

Bank balances as of March 1, 1954
Evanston State Bank and Trust Co.

General Fund	$1,362.74	
Endowment savings account	382.84	
Lincoln Bank and Trust Co. of Louisville (as of March 15)	5,124.10	

Evanston books and statements audited by Frank J. Orland.
Louisville books and statements audited by Henry M. Wilbur.

Financial Report of the Publication Committee, Business Manager, J. Frank Hall, Indianapolis

Bank balance, Jan. 1, 1953	603.94	
Receipts from back issues of JDR and biographical directory	137.40	
Total		$741.34

Disbursements

Office expense for 1952	$110.74	
Dues, American Association for Dental Editors	10.00	
Translations	10.00	
Storage	68.28	
Express and freight	17.89	
Mimeographing and supplies	19.60	
Clerical	50.00	
Postage and service charges	33.00	
Bank service charge	0.35	
Total		$319.86
Bank balance, Dec. 31, 1953	$421.48	

No auditor's report filed.

D. SATURDAY NIGHT GENERAL SESSION

The evening's exercises were opened by President Arnold by the reading of a letter from Dr. William J. Gies. In this letter read by Dr. Hatton, Dr. Gies regretted his inability to be at this meeting and sent his warmest greetings to the members. The Secretary-Treasurer was instructed to thank Dr. Gies for his message and wish him well. Dr. Paul C. Kitchin on behalf of the Novice Award Committee announced that the winner this year is Dr. Leo Korchin of Georgetown University, who could not be present to receive the award and the congratulations of the Association. Dr. Leonard S. Fosdick

described his recent trip to the Bushman country of South Africa, generously illustrated by colored slides. A charming and delightful presentation by students from the Bloomington campus of the University of Indiana closed the program.

E. CLOSING SESSION, SUNDAY AFTERNOON, MARCH 21, 1954

The meeting was opened for business by President Arnold, and Russell W. Bunting was called to address the meeting. He reported: 1. That he had represented the Association (specifically the North American divisions) in the preparation and operation of the program of Sub-section N at the holiday meeting of the American Association for the Advancement of Science, and that our President-Elect Paffenbarger had just received information that a new section of the A. A. A. S. was being organized for dentistry and would replace the Sub-section N. 2. That he had just returned from the recent Washington conference on civilian defense and that he was most gravely impressed by the serious portent and implications of our current situation and to urge all possible cooperation with the general and local machinery of civilian defense. The tellers reported a poll of 299 ballots and the list of officers elected for the 1954-1955 association year by a nearly unanimous vote. The Editor submitted a report on the proceedings of the meeting of the Board of Editors, including authorization to negotiate a new contract with The C. V. Mosby Co., and to solicit ethical donorships of an annual character for the support of the JOURNAL OF DENTAL RESEARCH. On behalf of the Council Dr. Paffenbarger reported that the date of the 1955 meeting had been set for March 18-20 and the place, Hotel Morrison in Chicago. Furthermore the Council reported that the dues assessment for the 1955 calendar year had been set at $10.00 per dues-paying member. The activities of the Council in addition to those previously mentioned were reported and approved. The persons listed elsewhere in these proceedings (p. 725) and as approved by the Council were formally elected to membership after unanimous suspension of the rules and the casting of a single marked ballot. The State Bank and Trust Co., of Evanston and the Lincoln Trust and Savings Bank of Louisville were approved for the new Association year for routine banking use. Four officers were certified for the business of signing checks and withdrawal orders of funds of the Association, namely: George C. Paffenbarger, Paul E. Boyle, Edward H. Hatton, and Dan Y. Burrill and two of these signers to be attached to each check or withdrawal order. Council was authorized to act for the Association in the recess between the 1954 and 1955 meetings. Letters of appreciation were ordered written to Dean Hine and President Wells of the University of Indiana for the fine entertainment supplied on Saturday night and for the excellent projecting service supplied by Mr. Richard Scott.

The list of the names of those who passed out of membership by death were read and the audience stood momentarily in recollection of their great services to dentistry and this Association. This list is printed elsewhere.

The officers elected for the 1954-1955 year were then called to face the presiding officer and duly installed. Following this the new President, George C. Paffenbarger, read his inaugural address, at the close of which, there being no further business, the 1954 meeting was adjourned sine die.

F. BOARD OF EDITORS

The Board of Editors met on March 19 and 20. The Editor discussed the correspondence with The C. V. Mosby Company in which they explained the annual deficit in publication of the JOURNAL and their inability to continue publication at a deficit. The Mosby Company proposed that the annual payment of $1,800 be deleted from the contract. A proposed plan to contract firms who manufacture dental equipment and supplies, and to offer them the oportunity to aid in the support of research was outlined. The effectiveness of this plan for *Chemical Abstracts* was explained by Dr. Lefkowitz and for the *Journal of Experimental Medicine and Biology* by Dr. Butcher. The possibility of the American Dental Association publishing and owning the JOURNAL was discussed. After considerable discussion at the second session the following motion of Dr. Zander, seconded by Dr.

Meisel, was passed, unanimously: "The Board of Editors recommends that the International Association for Dental Research authorize the Publication Committee to contact selected individuals, groups, corporations, or foundations and extend an invitation to support, in part, the JOURNAL OF DENTAL RESEARCH by annual contributions to the Association. Such contributions shall be acknowledged by a listing in the first section of each issue of the JOURNAL and in the program of the annual meeting. These contributors shall be known as 'Supporting Associates.' An annual certificate of appreciation shall be sent from the International Association for Dental Research to each supporting Associate.''

Dr. Dean explained the American Dental Association's proposal but no action was taken on this matter.

A proposal was received from the Washington Section, through Dr. Hampp, to make the full text of abstracts available before the meeting in a separate volume. It was suggested that this might increase interest and attendance, release an entire issue of the JOURNAL, attract more papers, be self-supporting, and improve the caliber of abstracts. This proposal was received for further study. Dr. Hall emphasized the value of keeping all back issues of the JOURNAL now in storage.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

AD INTERIM REPORT OF THE PRESIDENT

Geo. C. Paffenbarger, Senior Research Associate, American Dental Association Research Fellowship, National Bureau of Standards, Washington, D. C.

Your President believes that members of the International Association for Dental Research are not sufficiently acquainted with the functioning of the Association. He proposes to report to you from time to time, if feasible, in the JOURNAL OF DENTAL RESEARCH.

LOCAL ARRANGEMENTS COMMITTEE

A committee on local arrangements has been appointed, with Dr. Frank J. Orland, Walter G. Zoller Memorial Dental Clinic, The University of Chicago, Chicago 37, Illinois, as chairman. Other members of the committee are Dr. R. Quentin Blackwell, Dr. Frank M. Wentz, Dr. Leo M. Sreebny, Dr. Sholom Pearlman, and Dr. Joseph M. Gowgiel.

The Morrison Hotel is the headquarters hotel in Chicago for the 1955 meeting.

COMMITTEE ON THE SELECTION OF A MEETING PLACE FOR 1957

Dr. Joseph F. Volker, Dean, School of Dentistry, University of Alabama, Birmingham, Alabama, is chairman of this committee; the other two members are Dr. Ned B. Williams and Dr. William J. Simon. In odd-numbered years the IADR selects the meeting place; in even-numbered years the American Association of Dental Schools selects it.

COMMITTEE ON MEMBERS AND DIVISIONS OUTSIDE NORTH AMERICA

Dr. Geo. C. Paffenbarger, American Dental Association Research Fellowship, National Bureau of Standards, Washington 25, D. C., is chairman; the other two members are Dr. Paul E. Boyle and Dr. Robert G. Kesel. This committee was instructed by the Council of the IADR to make recommendations regarding the collection of dues from members residing outside North America. The committee recommended that such members be billed for the full amount of dues in those countries whose policy permits the exportation of currency for membership in technical societies. The Council accepted this recommendation and Dr. Dan Y. Burrill, the Assistant Secretary-Treasurer, is attempting to establish which countries permit their nationals to send dues abroad.

COMMITTEE ON ORGANIZATION AND ADMINISTRATIVE PROCEDURES

This committee consists of Dr. J. Roy Blayney, Chairman, Walter G. Zoller Memorial Dental Clinic, The University of Chicago, Chicago 37, Illinois; Dr. H. Trendley Dean; and Dr. Dan Y. Burrill. Its express purpose is to review the Constitution and By-

laws of the Association and make recommendations for any necessary revision. Since the revision of 1940, the growth of the organization and the international changes have made the administration of the Association more complex, so a revision may be in order.

COMMITTEE ON NOVICE AWARD

Dr. Samuel M. Gordon, when he was secretary of the Council on Dental Therapeutics of the American Dental Association, edited a very interesting and worth-while book entitled *Dental Science and Dental Art*, published by Lea & Febiger in 1938. At the LADR meeting in 1940, Dr. Gordon and the contributors to this volume offered the income from it to the Association to establish grants-in-aid in dental research. In 1948, using the income from the royalties of the book, the Council authorized the establishment of an award to be called the Dental Science and Dental Art Award, which has been unofficially shortened to Novice Award. The stipend is $100.00. The first of these awards was presented in 1953 to John J. Salley for his presentation, "Experimental Carcinogenesis in the Cheek Pouch of the Syrian Hamster." This research was carried on in the Division of Dental Research, School of Medicine and Dentistry, University of Rochester. The second award (1954) went to Major Leo Korchin, DC, USA, for the report, "Investigation to Determine the Effects of Starch Sponge Implanted in Bone." Major Korchin was a graduate student in the School of Dentistry, Georgetown University.

The present committee to select the recipient for the 1955 Dental Art and Dental Science Award is as follows: Dr. Paul C. Kitchin, Chairman, School of Dentistry, Ohio State University, Columbus 10, Ohio; Dr. Thomas J. Hill; Dr. Harold C. Hodge; Dr. H. Trendley Dean; and Mr. W. T. Sweeney.

COMMITTEE ON MEMBERSHIP

This committee consists of Dr. Ned B. Williams, School of Dentistry, University of Pennsylvania, Philadelphia 4, Pennsylvania, as chairman; Dr. Reidar F. Sognnaes; and Dr. Frank J. McClure. The chief function of this committee is to review the nominations for membership and report to the Council. Nomination forms and instructions should be obtained from Dr. E. H. Hatton, Secretary-Treasurer, 2419 Simpson St., Evanston, Illinois. The nominations should be forwarded to the chairman of this committee.

COMMITTEE ON INCORPORATION

By action of the Council at the 1954 meeting, the Board of Trustees was charged with investigating the feasibility of incorporating the Association and presenting a recommendation at the 1955 meeting. Suggestions relating to incorporating the Association should be forwarded to Dr. Thomas J. Hill, Institute of Pathology, Western Reserve University, Cleveland 6, Ohio, who is Chairman. Other trustees are Dr. H. Trendley Dean, Dr. Harold C. Hodge, Dr. Paul C. Kitchin, and Dr. L. M. Waugh.

PROGRAM COMMITTEE

The Program Committee consists of the President (Paffenbarger) as chairman, the President-elect (Boyle), and the Secretary (Hatton), as the Constitution provides. The chairman, by authority of the Council, appointed the following advisors to the Program Committee: Volker (Vice President); Burrill (Assistant Secretary-Treasurer); Robinson (Editor); Orland (Chairman, Local Arrangements Committee); and Mahler (Secretary, Dental Materials Group).

By mail ballot the committee and advisors have decided: (1) that printed abstracts of the papers to be given shall be made available prior to the meeting; (2) that titles and abstracts received after the deadline of Dec. 1, 1954, shall be automatically placed in an unclassified miscellaneous section in the order of receipt; (3) that four sections for the scientific sessions shall be used; two sections on classified subjects, one unclassified section for titles received after the deadline (the titles to appear in order of receipt), and one section on dental materials; (4) that there shall be one general scientific session at which the Dental Materials Group will be responsible for the program; (5) that a registration

fee of $2.00 shall be charged, this fee to include the printed abstracts; (6) that the Program Committee suggest to the Secretary and Assistant Secretary that some provision for early registration by mail shall be made possible; (7) that a reception and dinner on Saturday evening, March 19, 1955, will be held.

There is to be a joint evening session with the American Association of Dental Schools on Sunday evening, March 20, 1955, at 8 o'clock. The speaker will be Dr. Willard Frank Libby, noted radiochemist of the University of Chicago, who will speak on his epoch-making discovery that C^{14} determinations could be made a useful and accurate tool in dating the history of the world up to 25,000 years ago.

Members who desire to make recommendations pertaining to the functions of any committee should do so directly to the Committee Chairman with copies to the President and Secretary.

VII. OFFICERS AND MEMBERS ELECTED

ACTIVE OFFICERS: President—*George C. Paffenbarger,* National Bureau of Standards. President-elect—*Paul E. Boyle,* University of Pennsylvania. Vice-president—*Joseph F. Volker,* University of Alabama. Secretary-treasurer—*Edward H. Hatton,* Northwestern University. Assistant Secretary-treasurer—*Dan Y. Burrill,* University of Louisville. Trustee for a five-year term—*Harold C. Hodge,* University of Rochester. Editor—*Hamilton B. G. Robinson,* Ohio State University. Members of the Publication Committee—*J. Frank Hall,* Indiana University, and *Paul E. Boyle,* University of Pennsylvania.

MEMBERS ELECTED. FROM NORTH AMERICA: Illinois—*Frank C. Besic,* University of Chicago; *E. Lloyd Dubrul* and *Daniel Laskin,* University of Illinois. Louisina—*Harold M. Fullmer,* Loyola University. Maryland and District of Columbia—*Douglas M. Beebe, Jack L. Hartley, Donald C. Hudson, and James A. Mitchel,* National Bureau of Standards; *Arden Howell, Jr., James J. Kennedy, Harold J. McCann,* and *Robert R. Omata,* National Institute of Dental Research; *Ernest Esch Davies, Warren John Hedman,* and *J. L. Nemes,* National Navy Medical Center; *Donald Emerson Shay,* University of Maryland. Massachusetts—*William A. Curby* and *Gerald Shklar,* Tufts College; *Veikko Hurme,* Forsyth Dental Infirmary. Michigan—*Kamal Asgarzadeh,* University of Michigan; *Norman H. Rickles,* Percy Jones Army Hospital. Minnesota—*Karl H. Bruce* and *H. Quentin Royer,* Mayo Clinic; *Robert Earl Sausen* and *Joseph Chock Tam,* University of Minnesota. Missouri—*Norman H. Olsen* and *Genevieve D. Roth,* University of Kansas City. New York—*Austin H. Kutscher,* Columbia University; *Paul Benzer, Louis Robert Burman,* and *Harry Roth,* New York University; *Robert Lyndsay Campbell, John J. Salley, Henry W. Scherp,* and *David W. Smith,* University of Rochester. Ohio—*Steve Kolas* and *Arthur W. Radike,* Ohio State University; *Morris M. Matt,* Western Reserve University. South Carolina—*Benton Kutler,* Dental Clinic, Fort Jackson. Texas—*Bertram Eichel,* School of Aviation Medicine; *Martin Cattoni* and *David A. Hagerman,* University of Texas. Toronto, Canada—*R. M. Grainger,* University of Toronto. Total from North America, 43.

MEMBERS ELECTED FROM COUNTRIES OUTSIDE OF NORTH AMERICA: Australia—*John Heath.* Great Britain—*D. Jackson, G. A. Lammie, K. Little, Lady M. Mellanby, H. G. Radden, N. B. Symons, S. M. Weidman.* Germany—*Hans Diedrich Cremer, Josef Eschler, Carl-Heinz Fischer, Theo. Spreter von Freudenstein, Heinrich Ernest Hammer, Erich Hausser, Hans Heusser, Alfred Kantorowicz, Hermann Mathis, H. von Thiel, Konrad Thielman.* Italy—*Carlo Zerosi.* Japan—*Kei Enoki, Yoso Imagawa, Tadao Kirino, Seiichi Matsumiya, Tasuku Mimura, Gen Minoguchi, Iwao Nagai, Kazuo Nagai, Masao Onisi, Rokuro Suhari, Kenasaku Suzuki, Shinjiro Takahashi, Mamoru Yamada, Kiyoshi Yamasaki, Taro Yao, Seizo Yokota.* Norway—*Viktors Kalnins.* South America—*Alexander F. Baranoff, J. M. Frankel, Juan Carlos Muracioli.* Sweden—*Sten Ingre Ericcson.* Total from outside of North America, 41.

Total New Members Elected, 84.

NECROLOGY. *Paul H. Belding,* Waucoma, Iowa. *Robert E. Brawley,* Dayton, Ohio. *Thaddeus P. Hyatt,* Stamford, Conn. *Ernest F. Inskipp,* Dental School, College of Physicians

and Surgeons, San Francisco. *Ervin Mansbach*, Tel Aviv, Israel. *T. W. Osborn*, Johannesburg, South Africa. *L. E. Van Kirk*, University of Pittsburgh, Pittsburgh, Pa.

MEMBERS IN ATTENDANCE AT ONE OR MORE SESSIONS, LISTED BY SECTIONS

UNITED STATES

Ann Arbor—Bunting, Crowley, Henry, Jay, Kerr, Mahler, Peyton, Ramfjord. *Baltimore*—McCrea, Robinson. *Birmingham*—Bailey, Finn, Gibilesco, Klapper, Pigman, Schneyer, Volker, Wuehrmann. *Boston*—Berk, Cohen, Greep, Lisanti, Manly, Munson, Schiere, Sognnaes, Yurkstas. *Chicago*—R. Q. Blackwell, Blayney, Brodie, Burstone, Calandra, Carter, E. D. Coolidge, T. Coolidge, Dean, Doty, Engel, Ferguson, Fosdick, Freeman, Gans, Hatton, Hill, Jarabak, Kesel, Koser, Lasater, Medak, Orland, Pearlman, Rapp, Romnes, Schour, Schram, Skinner, Sreebny, Teuscher, Tilden, Tylman, Wach, Wallace, Wentz. *Cleveland*—Bruckner, Burwasser, Chase, Dressel, Gillis, Hill, Kimball, Kniesner, Wylie. *Columbus*—Kitchin, Lefkowitz, Permar, Robinson, Spangenberg. *Indianapolis*—Hall, Hine, Muhler, Phillips, Shafer, Swartz, Swenson, Van Huysen. *Iowa City*—Easton, Fisher, Simon, Smith, Wessels. *Kansas City*—Moore. *Louisville*—Burrill, Wilbur, Wolfe. *Minnesota*—Armstrong, Clark, Crawford, Lovestedt, Mitchell, Singer, Stafne, Zander. *Nebraska*—Hooper, Keys. *New York*—Butcher, Krasnow, Levy, Neumann, Siegel, Trautz. *Pacific Northwest*—Noyes. *Philadelphia*—Boyle, Chilton, Crowell, Grossman, N. O. Taylor, Williams. *Pittsburgh*—Cox, Leung, Meisel, Swanson. *Richmond*—Coy, Kreshover, Lyons. *Rochester*—Afonsky, Avery, Bibby, Brudevold, Hein, Hodge, Johansen. *San Francisco*—Agnew, Epstein, Fleming, Scrivener. *St. Louis*—Allen, Boling, Jasper, Gurley, Main. *Washington*—Arnold, Brauer, Burnett, Caul, Dickson, English, Forscher, Forziatti, Hampp, Harris, Hess, Knutson, Kruger, Likens, Losee, Ludwick, McCarthy, Nelsen, Paffenbarger, Person, Rault, Scott, Denton L. Smith, Stanford, Stephan, Sweeney, Duane F. Taylor, Thomas, Zipkin.

Not Members of a Section—Brumfield, Coleman, Dummett, T. E. Fischer, Harold S. Fleming, M. A. Goldberg, Haldi, Hayes, Knudtzon, Manhold, Molnar, T. E. Moore, Nelsen, Palmer, Ryge, Smyd, Strader.

CANADA

Toronto—Ellis, Grainger, Hunter, MacDonald, Nikiforuk, Rae.

FOREIGN

Stack, Trauner, Waerhaug.

NEW MEMBERS

Asgarzadeh, Besic, Curby, Eichel, Fullmer, Hartley, Hudson, Hurme, Kennedy, Kolas, Kutler, Laskin, Matt, Mitchell, Olsen, Radike, Genevieve Roth, Salley, Sausen, Scherp.

Total members, including those recently elected, 215. Guests, 214. Total Attendance, 429.

MEMBERS VOTING AT THE ANNUAL MEETING

Afonsky, Agnew, Alvin Aisenberg, M. S. Aisenberg, George Anderson, Arnim, Arnold, Bagnall, Backer-Dirks, Bailey, Claude Baker, Bauer, Becks, Berger, Bernier, Beube, Bevilacqua, Bhaskar, Blackerby, R. E. Blackwell, R. Quentin Blackwell, Blayney, Blumenthal, Bodecker, Bradley, Brand, Brashear, Broadbent, Brodie, Brodsky, Brucker, Brudevold, Burket, Burrill, Burstone, Butcher, Cahn, Canby, Carr, Carter, Caul, Chernausek, Cheyne, Chilton, Clough, M. Michael Cohen, E. D. Coolidge, Costich, Cox, Coy, Crawford, Crowley, Dahlberg, Dale, Darlington, Davis, Dawson, Dean, Dickson, Dietz, Docking, Dobbs, Dreizen, Dressell, Dummett, Easlik, Easton, L. F. Edwards, R. W. Edwards, Eggers-Lura, Elliott, Ellis, Erikson, Everett, Finn, A. B. Fisher, Theo. E. Fisher, Fitzgerald, Harold Fleming, W. C. Fleming, Fosdick, Fox, Freeman, Frey, Fritz, Gabel, Gafafer, Gibilesco, Gilchrist, Gilda, Gilkison, Gilmore, Ginn, Glass, Glasson, Goldsworthy, Gottsegen, Grainger, Greep, Grosby, Grossman, Gruebbel, Gurley, Hadjimarkos, Hadley, Hahn, Haldi, Harrison, Hatton, Hayes, Hein, J. L. Henry, Hess, Hildebrand, I. N. Hill, T. J. Hill, Hine, Hockett, Hodge, Hoffman, Holst, Hooper, Horowitz, Hughes, Hunter, Ireland, James, Jasper, E. L.

Johnson, L. G. Jordan, W. A. Jordan, Kaiser, Kerr, Kesel, D. A. Keys, Kitchin, Klaffenbach, Klapper, Edward Klein, Kniesner, Knighton, Knudtzon, Koser, Krasnow, Kraus, Kreshover, Krogh, Krogman, Kruger, Kurth, Kram, Leonard, Leung, Levy, Lieban, Lieber, Lisanti, Lovestedt, P. C. Lowery, R. A. Lowry, Mahler, Main, Mandel, Manly, Margolis, Marshall-Day, Massler, Medak, Mercado, Merkeley, Merritt, Millhon, Moen, Mitchell, Montgomery, Mary Moore, N. A. Moore, T. E. Moore, Anna Morse, Munson, Muhlemann, Hugh I. Myers, Raymond E. Myers, McCauley, McClure, McCollum, McCrea, Nelsen, Clyde A. Nelson, Neumann, Neuwirth, Nikiforuk, H. J. Noyes, Nygardöstby, Oartel, Ockerse, Oren A. Oliver, Orland, Ostrom, Paffenbarger, Palazzi, Parke, Pearlman, Pedersen, Permar, Person, H. W. Peterson, W. A. Peterson, Peyton, Phillips, Pindborg, Pigman, Pincus, Podolin, Radusch, Ramfjord, Rankow, Revesez, Rizzo, H. B. G. Robinson, Leonard Robinson, Rogers, Rosenstein, Roth, Rushton, Ryder, Ryge, Salzman, Savara, Schlack, Schneyer, Schour, Schroff, D. B. Scott, Seltzer, Shafer, J. C. Middleton Shaw, J. H. Shaw, Shell, Siegel, Simmonds, Simmons, Simon, Leon Singer, Skillen, Edwin L. Smith, Smyd, Sognnaes, Sorrin, Sprague, Sreebny, Stafne, Georg Stein, Suher, Swaney, H. A. Swanson, W. F. Swanson, Swartz, Taber, Norris O. Taylor, Telford, Teuscher, Thoma, B. O. A. Thomas, G. Earl Thompson, John R. Thompson, Harry S. Thomson, Tiblier, Tiecke, Trautz, Tylman, Van Huysen, Volker, Volland, Wach, Waggener, Walter, Warner, Waugh, Weber, Weinmann, Wentz, Wessels, Wheeler, Walter L. White, Wayne T. White, Wilbur, Ned B. Williams, Willman, Wm. R. Wolfe, Wuehrmann, W. L. Wylie, Wendell L. Wylie, Wynn, Young, Zander, Zipkin. Total, 296.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the British Section

School of Dental Surgery, University of Birmingham, Birmingham

April 2nd and 3rd, 1954

Compiled by A. E. W. Miles, Editor, British Section

Index of Participants and Sequence Numericals for Corresponding Abstracts

Bennett, D. T., 14; Bradford, E. W., 11; Farmer, E. D., 4; Fearnhead, R. W., 13, 15; Glasstone, S., 2; Hardwick, J. L., 3; Hartles, R. L., 1, 16; Hodson, J. J., 8; Jenkins, G. N., 12, 18; Leaver, A. G., 16; Miles, A. E. W., 15; Miller, J., 6, 17; Newman, P. A., 3; Prophet, A. S., 10; Speirs, R. L., 12, 18; Symons, N. B. B., 5; Tonge, C. H., 7; Turner, E. P., 9; Wasdell, M. R., 1.

The British Section of this Association held its second meeting at the Medical School, University of Birmingham, on the afternoon of Friday, April 2 and Saturday morning, April 3. Nearly forty members and a number of visitors attended and fifteen papers, most of which were in the nature of preliminary reports on work in progress, were read. On the evening of the first day vigorous discussions started during the afternoon session were continued more informally over dinner at the University Staff Club. There can be no doubt that this newly formed British Section is flourishing and is providing a valuable means for dental research workers to become acquainted personally and with one another's work.

1. The Fructofuranosidase (Invertase) Activity of Whole Saliva, *R. L. Hartles and Marie R. Wasdell, Biochemistry Department, School of Dental Surgery, University of Liverpool.* Observations upon the metabolism of glucose, fructose, maltose, and sucrose by resting saliva have shown that the disaccharides are metabolized as rapidly as their component sugars. Thus a knowledge of any glycosidases contained in the oral environment is essential for the comprehension of sugar utilization by the oral flora. Examination of the early reports upon the invertase activity of saliva show that these are based on necessarily inconclusive procedures. Our experiments are directed toward resolving this problem: by manometric measurement of the extent to which different glycosides are metabolized by whole saliva; and by determination of the enzymic content of fractionated saliva. While sucrose and raffinose {1- [(6-α-D galactopyranosyl)-α-D glucopyranose] -β-D fructofuranoside} are metabolized by the oral flora, melibiose (α-D galactosidoglucose) is not, thereby indicating that whole saliva contains a β-fructofuranosidase. A strain of lactobacillus studied in our laboratory produces acid rapidly from glucose and some other monosaccharides, but not from sucrose, raffinose, and maltose in the relatively short periods required for manometric experiments. The addition of saliva sterilized by Seitz filtration to washed suspensions of this bacillus did not promote acid production from sucrose or raffinose, as did similarly treated saliva to which invertase (British Drug Houses) has been added. These experiments suggest that certain microorganisms present in saliva possess an intracellular β-fructofuranosidase but that this enzyme is absent from salivary secretions.

2. The Vascularisation of Normal and Grafted Tooth Germs. *Shirley Glasstone, Department of Anatomy, University of Cambridge.* There has been considerable controversy on the question of vascularisation of the enamel organ. The extent to which capillaries penetrate into the enamel organ during development of the tooth germ varies in different mammals. There is more or less general agreement that in the rodents, the formation of enamel is dependent on the presence of erythrocytes and blood vessels in the enamel organ. In the rat, mouse, and golden hamster this vascularisation takes place approximately one to three days before enamel forms. It was found that similar changes took place in tooth germs grown on the chick chorio-allantois, depending on the level of development. In teeth that had reached their maximum stage of development on the membrane and where there was a thick layer of dentin and enamel, most of the enamel organ had been replaced by host blood vessels and connective tissue. In teeth that were later in development, there was partial invasion by blood vessels, erythrocytes, and connective tissue depending on the amount of enamel formed. The enamel organ was unaltered in teeth which remained healthy but did not develop. From the similarity between the vascularisation of the enamel organ in normal tooth germs of rat, mouse and golden hamster and those grown on the chick chorio-allantois, it is concluded that this is a process controlled by factors intrinsic to the developing tooth germ.

3. Some Observations on the Incidence and Emergency Treatment of Fractured Permanent Anterior Teeth of Children. *J. L. Hardwick and P. A. Newman, School of Dental Surgery, University of Birmingham.* Since 1952, 608 fractured permanent anterior teeth in 403 children have been examined and treated; 364 fractures occurred in a twelve-month period in the mouths of 242 children. Data obtained from a dental examination of 1,800 school children suggest that in this twelve-month period about 50 per cent of such fractures occurring in the 160,000 Birmingham children 6 to 16 years old were examined.

A. *Age and Sex Incidence.* (Percentage of children involved.)

AGE GROUP	6 AND UNDER	7	8	9	10	11	12	13	14	15	16	TOTAL
Males	1.0	6.8	11.8	10.7	13.2	9.4	5.7	2.2	2.6	1.0	1.2	65.6
Females	1.2	3.2	8.8	8.2	4.5	2.6	2.2	1.2	1.0	0.5	1.0	34.4
Males and Females	2.2	10.0	20.6	18.9	17.7	12.0	7.9	3.4	3.6	1.5	2.2	

B. *Incidence According to Position of Fracture.*—Enamel only, 6.8 per cent; Coronal dentin, not involving pulp, 67.2 per cent; Coronal dentin, involving pulp, 22.7 per cent; Root fractures, 3.3 per cent.

The true incidence of fractures of enamel and of the root is undoubtedly higher because not all such fractures would be recognised by the patient or referred for treatment.

C. *Incidence According to Teeth Involved.*—Many children are especially prone to traumatic injuries of the teeth; 4 per cent of the children examined had received such injuries on more than one occasion. The age and sex incidence suggests that the liability is increased during periods of boisterous and reckless activity. Eighty-six per cent of the fractures occurred in mouths in which there was an undue prominence of the affected teeth, accompanied usually by a lack of contact between the affected tooth and the corresponding tooth in the other jaw; these abnormalities were frequently associated with open lips habit, mouth breathing, or a history of thumbsucking.

4. The Isolation and Identification of a Virus From Acute Ulcerations of the Oral Mucosa. *E. Desmond Farmer, School of Dental Surgery, University of Liverpool.* A virus was isolated from small vesicles on the gingiva or buccal mucosa of three patients and from the saliva of a fourth patient, all of whom presented the clinical signs and symptoms of acute herpetic gingivostomatitis. The patients' ages were between 19 and 27 years. The contents of the vesicles in three cases and 0.2 ml. of saliva in the fourth case, where no vesicles were present, were mixed with 0.5 ml. of phosphate buffered nutrient broth containing 100 units per milliliter each of penicillin and streptomycin, and inoculated onto the chorioallantoic membrane (CAM) of fertilized hen's eggs 10 to 12 days old. After two to three days' incubation, the establishment of the infection was evidenced by the appearance of pocks. The CAM was harvested, ground up, and, after centrifugation at 2,000 r.p.m. for ten minutes, the supernatant was passaged through further eggs to increase the adaptation of the virus to the egg medium. After ten successive passages, each virus was washed and neutralization tests were carried out using a known positive high titer herpetic antiserum. With all four viruses the number of pocks was considerably reduced by the serum diluted up to 1 in 200 and in two cases when diluted up to 1 in 2,000. Preliminary neutralization tests, using the sera taken from three of the patients in the early stages of the infection (five to ten days) and again during convalescence (21 to 39 days), showed that there was no marked increase in the antibody titer of the serum with the progress of the disease. Biopsy specimens were taken from three patients. Two showed acidophilic intranuclear inclusion bodies in the epithelial cells adjacent to the vesicles. Similar inclusion bodies were found within the pocks on the chorioallantoic membrane.

5. Histochemical Study of Developing Teeth in Rodents. *N. B. B. Symons, Dental School, University of St. Andrews, Dundee.* Rats from twenty days' fetal life to four days after birth and mice from one to eight days old were used, both incisors and molars being studied. Earlier work (*Proc. Anat. Soc. Gt. Brit. & Irel.* Feb., 1954) on alkaline phosphatase activity has been extended, again using the simultaneous coupling azo dye method. It is found that where the cells of the enamel organ show the morphologic picture associated with maturation of the enamel, the entire cytoplasm of the ameloblasts is intensely stained. Glycogen distribution has been studied by the periodic acid-Schiff method and by Pritchard's chromic acid-diamino silver technique, and checked by the use of diastase. The intense basophilia with pyroninmethyl green staining, absent after treating with ribonuclease, indicates that the odontoblasts and ameloblasts are closely concerned in the production of the matrix of dentin and enamel, respectively. The organic basis of dentin and enamel has been investigated by staining with toluidine blue for metachromasia, alcian blue, and the p.a.s. method.

6. Micro-radiographic Studies of Dentin. *J. Miller, Turner Dental School, University of Manchester.* Grenz-ray studies of thin ($\pm$ 40 μ) transverse ground sections of dentin from an unerupted deciduous incisor show the Tomes fibril as a radiolucent structure surrounded by an area which is more radiopaque than the intertubular dentin matrix. This area of radiopacity corresponds in size with the "translucent area" which can be observed surrounding the Tomes fibril when a ground or decalcified section is viewed with transmitted light. This translucent area is shown by examination with polarized light to be birefringent. These findings indicate that the translucent area surrounding the fibril is not a post-mortem shrinkage phenomenon nor is it an optical or sectioning artefact. The translucent zone is an area of higher calcification than the dentin matrix. The birefringence of the zone in-

dicates a higher degree of particle orientation or crystallinity than the matrix. It would appear from serial ground sections that the size of the fibril and of the translucent area vary inversely. The fibril is largest near the pulp and the translucent area is more evident near the amelodentinal junction. The combined diameter of the fibril and the translucent area does not appear to vary greatly through the main part of the dentin, apart from the arborisation of the dentinal tubules near the amelodentinal junction.

7. Observations on the Formation of the Floor of the Mouth. *C. H. Tonge, Department of Anatomy, Medical School, Newcastle-upon-Tyne.* The floor of the mouth was dissected on both sides in two newborn infants and thirteen human adults, seven being edentulous. Sagittal sections were prepared of seven others, three being edentulous. All the adult specimens showed a separation between the posterior and anterior part of the mylohyoid muscle. The posterior part arose from the mylohyoid ridge and was limited to the mandibular molar region. The anterior part arose by one or more slips from the area in front of this. The posterior fibres were inserted into the hyoid bone and median raphé, the anterior into the raphé. In two specimens the raphé was deficient in the mid-line of the mandible leaving a space filled with fascia. In the remainder the raphé was attached to the inner aspect of the mandible leaving a deficiency between the anterior edge of the muscle and the mandible on each side of the mid-line. The separation between the anterior and posterior parts of the mylohyoid was marked by a space of variable size providing an important lymphatic pathway from the oral cavity to the submandibular region and conveying branches of the submental vessels in most of the specimens. In the newborn the anterior part of the mylohyoid extended as far posteriorly as the second deciduous molar, the posterior part encroaching on the mandibular ramus to be related to the first permanent molar crypt. Examination of fifty-seven mandibles confirmed the findings of the dissections regarding the limitations of the mylohyoid ridge to the molar region.

8. Cysts of the Gingiva. *J. J. Hodson, Dental School, University of Sheffield.* A number of cystic degenerations of epithelial clumps have been found in the gingivae in human fetuses and in adults. All stages of proliferation, keratinisation, and cyst formation occurred in both the early dental lamina and in its remnants. The fate of the cysts depends on the degree, extent, and direction of their expansion. Some probably open on the surface, leaving clefts, others may be involved by the developing teeth. Some degenerate and disappear, the keratin and debris being digested by giant cells. In sections of hyperplastic gingiva from adults, it is impossible to distinguish morphologically between cross-cut prolongations of basal cells from the surface epithelium and developmental remnants. Columns one or two cells thick may proliferate from the surface, the ends of which may differentiate and form bulbous epithelial bodies which may lose their connection with the surface. Keratinous pearls and cysts may develop from them. Cysts as described by Ramfjord (*Oral Surg., Oral Med., Oral Path.* **6**: 516-533, 1953) and Ritchey and Orban (*Oral Surg., Oral Med., Oral Path.* **6**: 765-771, 1953), all traceable to the surface, were observed in a case of gingivitis showing a bismuth line, in hyperplastic palatal mucosa due to denture irritation, and in a granulomatous epulis. Cystic degeneration commencing in the center of large epithelial clumps may break through to the outer surface and form clefts, or divide the area into lobules. A further case was found in which a lateral cyst attached to a mandibular third molar, arose from the epithelial attachment.

9. The Cuticles Covering the Enamel Surfaces of Erupted Teeth. *E. P. Turner, Turner Dental School, University of Manchester.* In recently

erupted teeth the transparent amorphous inner layer of Nasmyth's membrane, obtained by immersing teeth in acid, is marked by outcrops of enamel prisms corresponding with the perikymata typical of the unworn enamel surface. By reflected light this cuticle exhibited interference effects, and a number of specimens were unstable in strong acid at room temperature. The amorphous cuticle, similar in appearance, which is present on the enamel surface of teeth erupted for several years, is either unmarked or irregularly marked by the enamel prisms. This is consistent with progressive loss of enamel surface detail as age advances, due to wear. This cuticle does not exhibit interference effects, it is stable in strong acid at room temperature and in teeth showing attrition it extends over the worn enamel surface; it has not been observed on exposed dentin. A partial denture carrying the crown of a "normal" molar from which the natural cuticle had been removed was worn in the mouth for periods up to sixty-three days. At the end of each period a cuticle was obtained similar in appearance and properties to the cuticle of adult erupted teeth. A preliminary investigation using histochemical methods and paper partition chromatography indicates that this cuticle contains a mucoprotein and traces of lipoid. These observations suggest that two membranes similar in appearance but different in origin have been confused by authorities who state that the primary cuticle remains on the enamel surface throughout life.

10. Caries of the Dentin. *A. S. Prophet, Turner Dental School, University of Manchester.* Sections of carious lesions show that the initial bacterial invasion is via the fibril of the odontoblast. Few bacteria produce enzymes capable of breaking down the organic matrix of dentin, and these are able to act only after the matrix has been decalcified. This suggests that decalcification is of primary importance in the carious attack on dentin. Carious dentin is found to be more resistant to enzyme attack than decalcified normal dentin; in the deepest parts of carious lesions only the matrix immediately surrounding the tubule shows this change but in the superficial parts the whole intertubular area is affected. There is evidence that carious dentin contains collagen similar to that in normal dentin and that the increased resistance to enzymes is due to combination with substances from the saliva. The complete breakdown of organic dentin is a late stage in dental caries.

11. Observations on the Teeth of Reptiles. *E. W. Bradford, Dental School, University of St. Andrews, Dundee.* The structure of the dental tissues of Reptilia differs considerably from those of mammals. Enamel is present in the teeth of all dentate reptiles and varies considerably in structure in different species. In *Crocodilus niloticus* and Varanidae, the enamel is of considerable thickness, is aprismatic, and granular, with well-marked incremental lines of growth, whereas in *Alligator mississipiensis* the enamel is limited to a surface layer of hyaline structureless tissue showing an incremental pattern only near to the cervix. In Ophidia the enamel is limited to an extremely thin layer which has not been previously described. Granular reptilian enamels present many lamellae, which differ radically from human lamellae in forming an integral part of the development pattern. The dentin is deposited solely in globular form which gives the appearance of a richly branching system of tubules. In transverse section the dentin appears identical in structure with that found in the more primitive mammals, such as the Insectivora and Rodentia. Cementum, which reaches its highest degree of specialisation where tooth attachment is by gomphosis, is present in all dentate reptilia though there are modifications in structure which are related to differences in function. In Crocodilia it is characterised by the presence of coarse fibre bundles and scattered lacunae with radiating canaliculi. In ankylosed teeth, the cementum contains lacunae but no orientated fibre bundles.

12. Some Observations on the Fluoride Concentration of Dental Tissues. *G. N. Jenkins and R. L. Speirs, Department of Physiology, King's College, Newcastle upon Tyne.* It has been found, even in teeth formed during residence in "low fluorine" areas, that the concentration of fluoride in enamel, removed either with a bur or by brief exposure to acid, from the extreme outer surface of the crowns of permanent teeth is about four to five times higher than in the deeper layers. This high concentration is not acquired by the surface after eruption, because it is present on unerupted teeth and, at least in "low fluorine" areas, is independent of age. This difference between the surface and the rest of the enamel was not present in the deciduous teeth tested. Calculus has, throughout its thickness, a concentration of fluoride higher even than that on the enamel surface of permanent teeth in the same mouth. Estimations of the fluoride in dentin and whole enamel from permanent teeth at different ages show that dentin acquires considerable fluoride throughout life (average concentration in teeth from low fluorine areas: age 13 years, 0.012 per cent; age 50 years, 0.05 per cent) and that enamel shows a smaller rise (average concentration at age 13 years 0.005 per cent; at age 50 years, 0.0085 per cent). The rise of fluoride concentration in dentin is partly caused by a steady increase in primary dentin (samples adjacent to enamel), but mostly by the high fluoride content of secondary dentin (samples adjacent to pulp). Deciduous teeth extracted one and one-half to two years after topical application of NaF do not have an increased fluoride concentration on the layer removed by acid. The layer removed by grinding has not yet been tested.

13. The Innervation of the Dental Pulp Studied by Means of the Plasmal Reaction. *R. W. Fearnhead, The London Hospital Dental School.* According to Liang (*Anat. Rec.* **99**: 511, 1947) treatment of tissues with Schiff's reagent provides a simple and reliable method for staining nerves and nerve endings. Failure of this method to reveal the finer branches of nerves in the pulp, however, suggested that axis cylinders are not stained. Study of this technique using histochemical methods on a variety of mammalian tissues strongly supports the view that Liang's technique is in fact a plasmal reaction. Tissues stained by Liang's method were compared histologically with similar tissues stained by known methods for axis cylinders and nerve endings, namely, Bielschowsky-Gros, De Castro, Holmes, Bodian, and methylene blue (Schabadasch). The observations of other workers that plasmal is confined to the myelin of medullated nerves were confirmed. The fact that Liang's method will only stain medullated nerves would appear to limit its usefulness, particularly as a means of investigating the innervation of dentin. The critical differentiation provided by the plasmal reaction is, however, of great value for the demonstration of the patterns of distribution of medullated nerves and fatty aldehydes in the pulp and elsewhere.

14. Observations on the Permeability of the Dental Tissues to Penicillin. *D. T. Bennett, The London Hospital Dental School.* The coronal pulp cavities of twenty-three caries-free teeth ranging from 35 to 59 years of age were enlarged by removing most of the dentin with burs. The crowns were stood vertically in agar and a solution of penicillin placed in the pulp cavities. When tested bacteriologically after twelve to twenty-four hours, no penicillin had penetrated to the outer enamel surfaces. Penicillin was placed in the enlarged pulp canals of roots, the extremities of which were sealed by rubber tubes secured with wire ligatures, leaving the intermediate one-third of the roots exposed. After intervals of twelve to seventy-two hours no penicillin was detected on the surface of the cementum. When, however, areas of cementum were removed with a bur the underlying dentin was found

to be freely permeable. Preliminary experiments in which more extensive root areas were tested by using a rigid clamping apparatus and rubber seals produced similar results. These findings are in accord with those of other workers who have employed dyes of molecular weights comparable with that of penicillin. Experiments with various sealing agents confirmed the findings of Atkinson (*Brit. D. J.* **83**: 204, 1947) that rubber connections under pressure provide the only satisfactory seal.

15. Post-Mortem Color Changes in Teeth. *A. E. W. Miles and R. W. Fearnhead, The London Hospital Dental School.* A pink coloration of the teeth has been observed in five human bodies in advanced stages of decomposition, three being bodies discovered two to three weeks after death, one four months after death, and one exhumed four years after orthodox burial. Two died from strangulation, two from carbon monoxide poisoning, and the other from drowning. Sections through the teeth showed that the pink coloration was confined to the dentin into which it extended outward from the pulp for varying distances. Hem compounds were demonstrated spectroscopically in the affected dentin and the tubules contained granular benzidine-positive material. No bacteria could be demonstrated in the dentinal tubules. It is probable that the pinkness is a natural post-mortem phenomenon due to the seepage into the dentinal tubules of a fluid containing hemoglobin or its degradation products produced by post-mortem decomposition and liquefaction of the pulp. It may be supposed that if this phenomenon was very common, inorganic iron compounds would be found in the dentin of teeth buried for very long periods. No inorganic iron salts could be demonstrated histochemically, however, in sections of teeth from ancient graves. In one specimen the pink teeth contained sufficient carboxyhemoglobin for a rough quantitative estimation to be made by direct examination of the intact crown surface with a Hartridge reversion spectroscope. Furthermore, it was found possible to make a similar quantitative estimation on thick ground sections with a high-power oil-immersion incident light objective in combination with the reversion spectroscope.

PAPERS READ BY TITLE

16. The Isolation of Thymine (2, 6-Diheto-5-Methylpyrimidine) From the Cementum of the Sperm Whale (Physetermacrocephalus). *R. L. Hartles and A. G. Leaver, Biochemistry Department, School of Dental Surgery, University of Liverpool.* During a study of the fluorescent compounds of teeth the organic material was extracted from whale cementum by refluxing with ethylene glycol containing 3 per cent KOH. The liquor obtained was acidified and extracted with ether. The ether extracts were separated into several fractions by adsorption chromatography. It was suspected that the final fraction contained a pyrimidine. Precipitation with $AgNO_3$ and decomposition of the silver complex with H_2S yielded about 10 mg. of crystalline material (from 200 Gm. cementum). Chemical tests, ultraviolet absorption spectra, and finally infrared spectra showed that the material was thymine (2, 6-diheto-5-methylpyrimidine). The pyrimidine fluoresces strongly in ultraviolet light, but can only be responsible for a small fraction of the total tooth fluorescence. There is some evidence to suggest that whale dentin also contains some of the same pyrimidine but in a very much smaller quantity.

17. Bi-Annual Treatment With Silver Nitrate, Sodium Fluoride and Copper Cement in the Prevention of Dental Caries. *J. Miller, Turner Dental School, Manchester.* The teeth of groups of children 3 to 15 years of age (total number 450) were treated bi-annually with either silver nitrate, copper cement, or 1 per cent sodium fluoride for at least three years. Two years after

the end of treatment neither silver nitrate nor sodium fluoride produced a reduction of the caries incidence rates as assessed by the DMF index. Three years' treatment with copper cement resulted in an over-all reduction of 20 per cent in the caries incidence rates. Where treatment failed to prevent or arrest carious lesions full conservative treatment was employed to control the caries. The copper cement group required 75 per cent less conservative treatment than the other groups to control the caries in the permanent teeth.

18. An Investigation of Possible Differences Between the Chemical Composition of Deciduous Teeth Calcified Before and During the 1939-1945 War. *G. N. Jenkins and R. L. Speirs, Department of Physiology, King's College, Newcastle-upon-Tyne.* Sognnaes (*Am. J. Diseases Child.* **75**: 792, 1948) and others have concluded that the influence of the war-time diet on caries incidence in children was partly caused by increased resistance of the teeth, rather than by local effects in the mouth. It seemed of interest to compare the chemical composition of dental tissues formed before and during the war in a limited number of teeth which were available from the appropriate age groups. The solubilities of the enamel surfaces have been compared and the enamel or dentin has been analyzed for carbonate, phosphorus, P/CO_3 ratio (Sobel, *Metabolic Inter-relations,* **4**: 261, 1952), total carbohydrate (*Egyedi, D. Items of Interest,* **75**: 113, 1953), and fluoride. No significant differences have been found. No qualitative differences in trace elements have been detected spectrographically, but quantitative work by this method is in progress.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

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1. Serological Studies on Herpetic Infections Following Trigeminal Section. *S. A. Ellison, R. Moses, C. A. Carton, and H. M. Rose, Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, N. Y.* Recrudescence of infection with the virus of herpes simplex frequently follows intracranial trigeminal section. Accordingly, a series of such patients were studied by serological methods to determine what, if any, relation existed between preoperative antibody levels and these recurrences. An opportunity also was afforded to study the antibody response to recurrent herpes. Twenty-three patients have been examined, fifteen of whom developed herpes. No relation between antibody level and recurrence was observed. The antibody response to infection was irregular.

2. The Origin of the Teeth in Amblystoma Punctatum. *Barnet M. Levy, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Horstadius zone 3-5 of the head neural crest of stage 15 salamander embryos, when transplanted to the flank of older embryos (stage 28) along with presumptive oral ectoderm and presumptive oral entoderm, will result in the formation of cartilage and teeth. None of these tissues alone will produce this effect.

3. Periodontal Fiber Retachment in Replanted Incisors of the Monkey. *E. E. O. Butcher and Robert S. Vidair, New York University College of Dentistry, New York, N. Y.*

4. The Control of Pulpitis by Means of Systemic Antibiotic Therapy. *Melvin H. Amler, New York University College of Dentistry, New York, N. Y.* Cases which would heretofore have been considered hopeless to treatment other than root canal therapy or extraction (advanced hyperemia and early pulpitis) were considered. They were characterized clinically by vague, intermittent pains which persisted from one-half to three hours. These pains were initiated spontaneously or by lowering the temperature (below 60° F.), or by changing the patient to a recumbent position. In sixteen cases assumed to be of bacterial origin due to deep caries (no exposures), antibiotic therapy was administered systemically for forty-eight hours commencing within two days of onset of pain. The rationale was to control the bacterial infection in the pulp by means of a systemic blood level of antibiotics before the pulp was damaged. Within one to three days after therapy was commenced the pain disappeared. Vitality measurements and x-ray studies up to eighteen months after the antibiotic treatment indicated no apparent abnormalities in the pulp for fifteen cases. In one case the pulp was nonvital after two months but no radiolucent area was visible. In four successful cases penicillin was used (200,000 units, buffered, long-acting tablets by mouth every six hours) and in the remaining cases Terramycin was used (250 mg. tablets every six hours). In six additional cases in which antibiotic therapy was commenced more than two days after the onset of the symptoms,

the pain was at no time alleviated and the pulps all developed suppuration. In these latter cases both penicillin and Terramycin were used, and antibiotic therapy was continued for ninety-six hours.

5. Experimental Formation of Dentin in the Pulps of Teeth. *Alexander Seelig, Dental Department, Montefiore Hospital, New York, N. Y.* To clarify the mechanisms of the formation of hard tissue in the pulp, a series of experiments was carried out on the teeth of rhesus monkeys. The response of the pulp to various drugs was studied under varying conditions of time and surgical technics. It has been determined that hard tissue formation in the pulp depends on the state of the pulp at the time of exposure, the reaction of the pulp to the materials introduced, and the method of operation. Six monkeys, ranging in age from 8 months to 4 years, were used, the observation therefore including the reactions of the pulps of young and old deciduous teeth and young permanent teeth. Histologic evidence indicates that healthy cells in a healthy environment are necessary for the formation of dentin. Under such circumstances, introduction of viable dentin chips serves as a nidus for formation of true dentin, which is dependent on the differentiation of new odontoblasts. A suitable base for secretion of true dentin must be provided, and care taken to avoid contamination of the preparation. It is possible for dentin to form at one point while the pulp has a localized abscess in another area.

6. Contributions to Tongue Pathology. *George Stein, School of Dental and Oral Surgery, Columbia University and the Montefiore Hospital, New York, N. Y.* (a) Histopathology of the geographic tongue. The biopsy of a case of geographic tongue (wandering rash) shows a superficial polymorphonuclear leukocytic infiltration of the epithelium and the lamina propria in the direction into which the lesion "wanders." The hornified layer is separated from the prickle cell layer, and the exposed surface of the malpighian layer is covered by fibrin and polymorphonuclear leukocytes. Healing takes place by redevelopment of the keratin layer with decrease and final disappearance of cellular infiltration. (b) Effect of vitamins on the repapillation of the tongue in nutritionally depleted patients. For therapeutic reasons, patients of the metabolic ward of Montefiore Hospital were kept on a low-vitamin diet, thus developing a multivitamin deficiency. The sequence and rate of the appearance of deficiency symptoms in the oral cavity and their disappearance after adding vitamins to the diet are demonstrated in serial pictures.